WEST

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starting with: MODIFI$(MODIFIER/MATRIX).P28-P86,P88-P88,P23-P27,P20-P22,P1-P18.

Search Results -

Terms	Documents
18 same (variant or mutant or modifi$)	6

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Refine Search: 18 same (variant or mutant or modifi$) Clear

Search History

Today's Date: 5/28/2001

DB Name	Query	Hit Count	Set Name
USPT,PGPB,JPAB,EPAB,DWPI	18 same (variant or mutant or modifi$)	6	L9
USPT,PGPB,JPAB,EPAB,DWPI	pullulanase.ti.	215	L8
USPT,PGPB,JPAB,EPAB,DWPI	l6 and (100 amino acids or 200 amino acids or 300 amino acids)	12	L7
USPT,PGPB,JPAB,EPAB,DWPI	l5 and (N terminal)	61	L6
USPT,PGPB,JPAB,EPAB,DWPI	l4 and (deletion)	77	L5
USPT,PGPB,JPAB,EPAB,DWPI	l3 and (mutant or modifi$ or variant)	233	L4
USPT,PGPB,JPAB,EPAB,DWPI	l2 and (Bacillus or Klebsiella)	277	L3
USPT,PGPB,JPAB,EPAB,DWPI	l1 and bacteria$	426	L2
USPT,PGPB,JPAB,EPAB,DWPI	pullulanase	1027	L1

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Search Results - Record(s) 1 through 6 of 6 returned.

☐ 1. Document ID: JP 10327868 A

L9: Entry 1 of 6 File: JPAB Dec 15, 1998

PUB-NO: JP410327868A
DOCUMENT-IDENTIFIER: JP 10327868 A
TITLE: MUTANT PULLULANASE

PUBN-DATE: December 15, 1998

INVENTOR-INFORMATION:

NAME	COUNTRY
SUMITOMO, NOBUYUKI	
HATADA, YUUJI	
ICHIMURA, TAKASHI	
SAITO, KAZUHIRO	
KAWAI, SHUJI	
ITO, SUSUMU	

INT-CL (IPC): C12N 15/09; C07H 21/04; C11D 3/386; C11D 7/42; C12N 1/21; C12N 9/00; C12N 9/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KMC | Draw. Desc | Image

☐ 2. Document ID: EP 1092014 A2, WO 200001796 A2, AU 9948971 A

L9: Entry 2 of 6 File: DWPI Apr 18, 2001

DERWENT-ACC-NO: 2000-160767
DERWENT-WEEK: 200123

TITLE: Variant bacterial pullulanases and isoamylases having, e.g. increased thermostability, used for converting starch from potatoes into high fructose syrup

INVENTOR: BISGARD-FRANTZEN, H; SVENDSEN, A

PRIORITY-DATA: 1998DK-0000868 (July 2, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
EP 1092014 A2	April 18, 2001	E	000	C12N009/44
WO 200001796 A2	January 13, 2000	E	116	C12N000/00
AU 9948971 A	January 24, 2000	N/A	000	C12N000/00

INT-CL (IPC): C12N 0/00; C12N 9/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Drawi Desc | Image

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☐ 3. Document ID: EP 1060253 A2, WO 9945124 A2, AU 9929801 A, BR 9908422 A

L9: Entry 3 of 6 File: DWPI Dec 20, 2000

DERWENT-ACC-NO: 1999-540851
DERWENT-WEEK: 200105

TITLE: New modified pullulanase for saccharification of aqueous liquefied starch

INVENTOR: MILLER, B S; SHETTY, J K

PRIORITY-DATA: 1998US-0034630 (March 4, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
EP 1060253 A2	December 20, 2000	E	000	C12N015/56
WO 9945124 A2	September 10, 1999	E	048	C12N015/56
AU 9929801 A	September 20, 1999	N/A	000	C12N015/56
BR 9908422 A	October 31, 2000	N/A	000	C12N015/56

INT-CL (IPC): C12N 1/21; C12N 1/21; C12N 9/34; C12N 9/44; C12N 15/56; C12N 15/75; C12P 19/16; C12R 1/10; C12R 1/22; C12N 9/44; C12R 1/07; C12N 1/21; C12R 1/10; C12N 9/44; C12R 1/07; C12R 1/22

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Drawi Desc | Image

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☐ 4. Document ID: JP 10327868 A

L9: Entry 4 of 6 File: DWPI Dec 15, 1998

DERWENT-ACC-NO: 1999-099031
DERWENT-WEEK: 199909

TITLE: New mutant pullulanase - useful in bleach-containing detergents

PRIORITY-DATA: 1997JP-0141596 (May 30, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 10327868 A	December 15, 1998	N/A	019	C12N015/09

INT-CL (IPC): C07H 21/04; C11D 3/386; C11D 7/42; C12N 1/21; C12N 9/00; C12N 9/44; C12N 15/09; C12N 15/09; C12R 1/07; C12N 1/21; C12R 1/19; C12N 9/44; C12R 1/19

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 5965442 A, JP 07177891 A, JP 2604988 B2

L9: Entry 5 of 6 File: DWPI Oct 12, 1999

DERWENT-ACC-NO: 1995-279919
DERWENT-WEEK: 199949

TITLE: Modifying a transferase by enhancing hydrophobicity of a selected site - increases transfer activity, also new mutant neo-pullulanase(s)

INVENTOR: KANEKO, H; KURIKI, T ; OKADA, S ; SHIMADA, J ; TAKADA, T ; TAKATA, H ; YANASE, M

PRIORITY-DATA: 1993JP-0306096 (November 12, 1993)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 5965442 A	October 12, 1999	N/A	000	C12N015/11
JP 07177891 A	July 18, 1995	N/A	018	C12N015/09
JP 2604988 B2	April 30, 1997	N/A	018	C12N015/09

INT-CL (IPC): C12N 9/44; C12N 15/09; C12N 15/11; C12N 15/54; C12N 15/56; C12N 15/70; C12N 9/44; C12R 1/19; C12N 15/09; C12R 1/07; C12N 15/09; C12R 1/07; C12N 9/44; C12R 1/19

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 4737459 A

L9: Entry 6 of 6 File: DWPI Apr 12, 1988

DERWENT-ACC-NO: 1988-119083
DERWENT-WEEK: 198817

TITLE: Amylase(s), pullulanase and ethanol prodn. - by culture of mutant strains of clostridium thermosulphurogenes and thermohydrosulphuricum

INVENTOR: HYAN, H H; ZEIKUS, J G

PRIORITY-DATA: 1985US-0716045 (March 26, 1985), 1984US-0652588 (September 18, 1984)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 4737459 A	April 12, 1988	N/A	015	N/A

INT-CL (IPC): C12N 9/34; C12P 7/14

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=> d his

     (FILE 'HOME' ENTERED AT 08:23:26 ON 28 MAY 2001)

     INDEX 'ADISALERTS, ADISINSIGHT, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS,
     BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT,
     CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE,
     DRUGB, DRUGLAUNCH, DRUGMONOG2, DRUGNL, ...' ENTERED AT 08:23:34 ON 28
MAY
     2001
               SEA PULLULANASE
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             132   FILE AGRICOLA
              18   FILE ANABSTR
              15   FILE AQUASCI
             161   FILE BIOBUSINESS
              14   FILE BIOCOMMERCE
             604   FILE BIOSIS
             552   FILE BIOTECHABS
             552   FILE BIOTECHDS
             254   FILE BIOTECHNO
             134   FILE CABA
               6   FILE CANCERLIT
            1286   FILE CAPLUS
             164   FILE CEABA-VTB
               2   FILE CIN
               7   FILE CONFSCI
               8   FILE DDFB
               4   FILE DDFU
             242   FILE DGENE
               8   FILE DRUGB
               5   FILE DRUGU
               1   FILE EMBAL
             302   FILE EMBASE
             127   FILE ESBIOBASE
              16   FILE FOREGE
             191   FILE FROSTI
             387   FILE FSTA
             116   FILE GENBANK
             146   FILE IFIPAT
             151   FILE JICST-EPLUS
             263   FILE LIFESCI
             243   FILE MEDLINE
               8   FILE OCEAN
             317   FILE PASCAL
               1   FILE PHIN
              18   FILE PROMT
             575   FILE SCISEARCH
              36   FILE TOXLINE
              93   FILE TOXLIT
             562   FILE USPATFULL
             300   FILE WPIDS
             300   FILE WPINDEX
L1             QUE PULLULANASE
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     FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, CAPLUS' ENTERED AT 08:25:31 ON
     28 MAY 2001
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L2	396	S L1 AND (MODIF? OR MUTANT OR VARIANT)
L3	1	S L2 AND (N TERMINAL DELETION)
L4	20	S L2 AND DELETION
L5	16	DUP REM L4 (4 DUPLICATES REMOVED)
L6	158	S L2 AND BACTERI?
L7	99	S L6 AND (BACILLUS OR KLEBSIELLA)
L8	30	S L7 AND (DERAMIFICANS OR PNEUMONIAE)
L9	21	DUP REM L8 (9 DUPLICATES REMOVED)
L10	4	S L9 AND (CDNA OR CLONE)

=> d l9 ibib ab 1-21

L9 ANSWER 1 OF 21 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2001:367036 CAPLUS
TITLE: Screening and production of debranching enzyme from **bacteria**
AUTHOR(S): Naito, Shinsuke
CORPORATE SOURCE: Department of Agriculture, Research Lab. for Microbiology, Meijo University, Japan
SOURCE: Meijo Daigaku Nogakubu Gakujutsu Hokoku (2001), 37, 103-108
CODEN: MDNGBZ; ISSN: 0910-3376
PUBLISHER: Meijo Daigaku Nogakubu
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
AB Debranching enzyme-producing **bacteria** were investigated widely from various sources using the following medium (1% maltose or 0.5% .beta.-limited dextrin (.beta.-L.D.), 0.2% (NH4)2HPO4, 0.1% sodium glutamate, trace of yeast exts. and other inorg. salts) and a slightly **modified** selective procedure for **bacteria** producing **pullulanase** and isoamylase by Ruben et al. This screening method was very effective in comparison with their report and consequently about 40% of isolated colonies (273 strains) yielded the enzymes. Most of them were supposed as **Klebsiella** species showing relatively high prodn. of **pullulanase** and no amylolytic activity. Some were supposed as **Bacillus** species which were found to produce both **pullulanase** and .alpha.-amylase in their culture fluid. On the other hand, 8 strains selected as isoamylase-producing **bacteria** showed a little activities on amylopectin as a substrate at pH 5.2 and 40.degree.C, but did not at 50.degree.C. Extracellular **pullulanase** activities of No. 5-4. No. 28, No. 18 and No. 65 supposed as **Klebsiella** sp. were 83, 70 and 64 nkat./mL of culture, resp. Acid- and temp.-tolerance of these enzymes were slightly superior ot that of K. **pneumoniae**. **Pullulanase** activity of No. G-17 supposed as **Bacillus** sp. was 41 nkat/mL of culture and acid-tolerance of its enzyme was inferior to that of B. acidopullulyticus.

L9 ANSWER 2 OF 21 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:68546 CAPLUS
DOCUMENT NUMBER: 132:104698
TITLE: Glucoamylase **variants** with improved specific activity and/or thermostability
INVENTOR(S): Nielsen, Bjarne Ronfeldt; Svendsen, Allan; Pedersen, Henrik; Vind, Jesper; Hendriksen, Hanne Vang; Frandsen, Torben Peter
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 117 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000004136	A1	20000127	WO 1999-DK392	19990709

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ,

DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS,
JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK,
MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ,
TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD,
RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK,
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9947699	A1	20000207	AU 1999-47699	19990709
EP 1097196	A1	20010509	EP 1999-931029	19990709

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO

PRIORITY APPLN. INFO.:

DK 1998-937	A	19980715
DK 1998-1667	A	19981217
WO 1998-DK937	W	19980715
WO 1998-DK1667	W	19981217
WO 1999-DK392	W	19990709

AB The invention relates to a **variant** of a parent fungal glucoamylase, which exhibits improved thermal stability and/or increased specific activity using saccharide substrates. The x-ray structure and/or model-build structure of Aspergillus awamori **variant** X100 glucoamylase was subjected to mol. dynamics simulations to identify regions important for temp.-stable activity. The truncated G1 glucoamylase from Aspergillus niger was **modified** by (1) random mutagenesis, (2) localized random, doped mutagenesis, or (3) PCR shuffling spiked with DNA oligonucleotides in order to prep. **variants** having improved thermostability compared to the parent enzyme. Such glucoamylase **variants** have use in starch saccharification, oligosaccharide prodn., specialty syrups, producing ethanol for fuel, producing beverages, and producing org. compds. (citric acid, ascorbic acid, lysine, glutamic acid).

REFERENCE COUNT: 4
REFERENCE(S):
(1) Chen, H; Protein Eng (ENGLAND) 1995, V8(6), P575 CAPLUS
(2) Fierobe, H; Biochemistry (UNITED STATES) 1996, V35(26), P8696 CAPLUS
(3) Iowa State University Research Foundation Inc; WO 9803639 A1 1998 CAPLUS
(4) Novo Nordisk AS; WO 9200381 A1 1992 CAPLUS

L9 ANSWER 3 OF 21 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:34954 CAPLUS
DOCUMENT NUMBER: 132:90065
TITLE: Genetic engineering of starch-debranching enzymes for improved thermostability and specificity
INVENTOR(S): Bisgard-Frantzen, Henrik; Svendsen, Allan
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000001796	A2	20000113	WO 1999-DK381	19990702
WO 2000001796	A3	20000309		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ,
DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS,
JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK,
MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ,
TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD,
RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9948971	A1	20000124	AU 1999-48971	19990702
EP 1092014	A2	20010418	EP 1999-932675	19990702

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRIORITY APPLN. INFO.:	DK 1998-868	A	19980702
	WO 1999-DK381	W	19990702

AB The invention relates to a genetically engineered **variant** of a parent starch-debranching enzyme, i.e. a **pullulanase** or an isoamylase, the enzyme **variant** having an improved thermostability at a pH in the range of 4-6 compared to the parent enzyme and/or an increased activity towards amylopectin and/or glycogen compared to the parent enzyme. Methods for producing such starch-debranching enzyme **variants** with improved thermostability and/or altered substrate specificity are provided. Alignment of **pullulanases** of **Bacillus** acidopullulyticus and **Bacillus deramificans**, and of isoamylases of Rhodothermus marinus and Pseudomonas amyloderamosa, identified specific loop regions and amino acid residues appropriate for substitution with thermostability-conferring residues. The **modified** enzymes should yield improved conversion of starch to one or more sugars.

L9 ANSWER 4 OF 21 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:	1999:577030 CAPLUS
DOCUMENT NUMBER:	131:196365
TITLE:	N-terminal-truncated analogs of **bacterial pullulanases** retaining normal enzymic activity
INVENTOR(S):	Miller, Brian S.; Shetty, Jayarama K.
PATENT ASSIGNEE(S):	Genencor International, Inc., USA
SOURCE:	PCT Int. Appl., 49 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9945124	A2	19990910	WO 1999-US4627	19990303
WO 9945124	A3	19991118		

W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9929801	A1	19990920	AU 1999-29801	19990303
BR 9908422	A	20001031	BR 1999-8422	19990303
EP 1060253	A2	20001220	EP 1999-911068	19990303

R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE, FI

PRIORITY APPLN. INFO.:	US 1998-34630	A	19980304
	WO 1999-US4627	W	19990303

AB **Pullulanases** from **Bacillus** and **Klebsiella** that retain normal 1,6-.alpha.-glycosidase activity despite having truncations of up to 300 amino acids from the N-terminal domain, optionally with further amino acid substitutions, and that may be useful in the starch industry are described. The present invention provides methods for producing the **modified pullulanase**, enzymic compns. comprising the **modified pullulanase**, and methods for the saccharification of starch comprising the use of the

enzymic compns. Expression of the **Bacillus deramificans** **pullulanase** gene in B. licheniformis hosts lacking the Carlsberg subtilisin and endopeptidase Glu-C resulted in the appearance of a series of N-terminal deletions of the **pullulanase**. Saccharification of starch with mixts. of glucoamylase (20%) and the **pullulanases** (80%) led to the saccharification of the starch without the formation of disaccharides.

L9 ANSWER 5 OF 21 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:282063 CAPLUS
DOCUMENT NUMBER: 130:316457
TITLE: Plaque-inhibiting oral compositions comprising enzymes
INVENTOR(S): Tsuchiya, Rie
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 25 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9920239	A1	19990429	WO 1998-DK452	19981016
W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9896213	A1	19990510	AU 1998-96213	19981016
EP 1023037	A1	20000802	EP 1998-949952	19981016
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				

PRIORITY APPLN. INFO.: DK 1997-1191 A 19971017
WO 1998-DK452 W 19981016

AB Oral compns. comprise plaque-inhibiting or plaque-removing enzymes, in particular at least one starch-hydrolyzing enzyme, e.g. an .alpha.-amylase or a debranching enzyme such as a **pullulanase**, and/or at least one starch-**modifying** enzyme, e.g. a transglucosidase or a CGTase, and to a method for inhibiting plaque formation or removing plaque using such oral compns. Hydroxyapatite coated with sterilized saliva were immersed in a culture broth contg. various microorganisms and 200MANU/mL Maltogenase (**bacterial** maltogenic .alpha.-amylase) so that an oral biofilm was formed on the disks. After cultivation, the disks were rinsed and stained and the intensity of the red color was compared to that of non-treated disks. The plaque intensity of the Maltogenase was 36.8 as compared with 100% for the controls.

REFERENCE COUNT: 11
REFERENCE(S):
(1) Aspro-Nicholas; GB 1284728 A 1972 CAPLUS
(3) Blendax-Werke R Schneider & Co; FR 7314 M 1969 CAPLUS
(4) Blendax-Werke R Schneider & Co; DE 1948468 A 1971 CAPLUS
(5) Harrisson; US 3194738 A 1965 CAPLUS
(6) Klueppel; US 5145665 A 1992 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 6 OF 21 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 19[redacted]259400 BIOSIS
DOCUMENT NUMBER: PREV199698815529
TITLE: Mutagenesis of Burkholderia pseudomallei with Tn5-OT182: Characterization of **mutants** deficient in protease, lipase and lecithinase.
AUTHOR(S): Deshazer, D.; Brett, P. J.; Woods, D. E.
CORPORATE SOURCE: Univ. Calgary, Calgary, AB Canada
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1996) Vol. 96, No. 0, pp. 176. Meeting Info.: 96th General Meeting of the American Society for Microbiology New Orleans, Louisiana, USA May 19-23, 1996
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

L9 ANSWER 7 OF 21 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 96:147063 SCISEARCH
THE GENUINE ARTICLE: TV358
TITLE: MOLECULAR MIMICRY - THE GEOGRAPHICAL-DISTRIBUTION OF IMMUNE-RESPONSES TO **KLEBSIELLA** IN ANKYLOSING-SPONDYLITIS AND ITS RELEVANCE TO THERAPY
AUTHOR: EBRINGER A (Reprint); AHMADI K; FIELDER M; RASHID T; TIWANA H; WILSON C; COLLADO A; TANI Y
CORPORATE SOURCE: UNIV LONDON KINGS COLL, DIV LIFE SCI, IMMUNOL SECT, CAMPDEN HILL RD, LONDON W8 7AH, ENGLAND (Reprint)
COUNTRY OF AUTHOR: ENGLAND
SOURCE: CLINICAL RHEUMATOLOGY, (JAN 1996) Vol. 15, Supp. 1, pp. 57-61.
ISSN: 0770-3198.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: CLIN
LANGUAGE: ENGLISH
REFERENCE COUNT: 24
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The discovery that HLA-B27 is linked to ankylosing spondylitis (AS) and HLA-DR1/DR4 to rheumatoid arthritis (RA) has provided new approaches to the study of the possible causation of these diseases.

Several theories have been proposed to explain these associations but only one, namely ''molecular mimicry'', has provided a specific aetiological agent for each of these diseases.

Molecular mimicry between HLA-B27 and two molecules in **Klebsiella** microbes: nitrogenase and **pullulanase** D has been reported whilst in Proteus microbes, the haemolysin molecule shows sterochemical similarity to HLA-DR1/DR4.

Elevated immune responses to **Klebsiella** microbes have been demonstrated in AS patients from 10 different countries and this wide geographical distribution suggests that the same aetiological agent is probably acting in producing this condition.

Furthermore RA patients show similar immune responses to Proteus microbes.

Whether AS or RA are caused by these **bacteria** can only be resolved by tissue typing all rheumatological patients early, in the course of their disease and then assessing their response to antibiotic chemotherapy in longitudinal studies involving double-blind crossover trials.

It is possible that in the future, the course of AS or even RA could be **modified** by adequate antibiotic chemotherapy or even diets which affect the substrates on which these **bacteria** grow.

L9 ANSWER 8 OF 21 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 95:636185 SCISEARCH

THE GENUINE ARTICLE: RU825
TITLE: E[redacted]ACELLULAR SECRETION OF **PULLULA[redacted]E** IS UNAFFECTED BY MINOR SEQUENCE CHANGES BUT IS USUALLY PREVENTED BY ADDING REPORTER PROTEINS TO ITS N-TERMINAL

OR

C-TERMINAL END

AUTHOR: SAUVONNET N; POQUET I; PUGSLEY A P (Reprint)
CORPORATE SOURCE: INST PASTEUR, MOLEC GENET UNIT, CNRS, URA 1149, 25 RUE DR ROUX, F-75724 PARIS 15, FRANCE (Reprint); INST PASTEUR, MOLEC GENET UNIT, CNRS, URA 1149, F-75724 PARIS 15, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: JOURNAL OF BACTERIOLOGY, (SEP 1995) Vol. 177, No. 18, pp. 5238-5246.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 32

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Linker insertions in the **pullulanase** structural gene (pulA) were examined for their effects on **pullulanase** activity and cell surface localization in Escherichia coli carrying the cognate secretion genes from **Klebsiella** oxytoca. Of the 23 insertions, 11 abolished **pullulanase** activity but none were found to prevent secretion. To see whether more drastic changes affected secretion, we fused up to five reporter proteins (E. coli periplasmic alkaline phosphatase, E. coli periplasmic maltose-binding protein, periplasmic TEM beta-lactamase, Erwinia chrysanthemi extracellular endoglucanase Z, and **Bacillus** subtilis extracellular levansucrase) to three different positions in the **pullulanase** polypeptide: close to the N terminus of the mature protein, at the C terminus of the protein, or at the C terminus of a truncated **pullulanase variant** lacking the last 256 amino acids. Only 3 of the 13 different hybrids were efficiently secreted: 2 in which beta-lactamase was fused to the C terminus of full-length or truncated **pullulanase** and 1 in which maltose-binding protein was fused close to the N terminus of **pullulanase**. Affinity-purified endoglucanase-**pullulanase** and **pullulanase**-endoglucanase hybrids exhibited apparently normal levels of **pullulanase** activity, indicating that the conformation of the **pullulanase** segment of the hybrid had not been dramatically altered by the presence of the reporter. However, **pullulanase** endoglucanase hybrids were secreted efficiently if the endoglucanase component comprised only the 60-amino-acid, C-terminal cellulose-binding domain, suggesting that at least one factor limiting hybrid protein secretion might be the size of the reporter.

L9 ANSWER 9 OF 21 MEDLINE
ACCESSION NUMBER: 95221318 MEDLINE
DOCUMENT NUMBER: 95221318 PubMed ID: 7706211
TITLE: Random mutagenesis of **pullulanase** from **Klebsiella** aerogenes for studies of the structure and function of the enzyme.
AUTHOR: Yamashita M; Kinoshita T; Ihara M; Mikawa T; Murooka Y
CORPORATE SOURCE: Department of Fermentation Technology, Faculty of Engineering, Hiroshima University.
SOURCE: JOURNAL OF BIOCHEMISTRY, (1994 Dec) 116 (6) 1233-40.
Journal code: HIF; 0376600. ISSN: 0021-924X.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950518
Last Updated on STN: 19970203
Entered Medline: 19950511

AB To study the structure and function of **pullulanase** from **Klebsiella** aeroge[redacted], a method involving random mu[redacted]genesis of the entire gene for **pullulanase** was used. Out of 50,000 clones screened at high temperature, seven genes for **mutant** proteins were identified by DNA sequencing. The amino acid substitutions in the seven **mutant** proteins were clustered on the NH2-terminal side of the four conserved regions found in alpha-amylases. These **mutant** **pullulanases** were classified into two types: those whose catalytic activity was altered and those whose thermal stability was increased. The results presented here and in previous reports suggest that **pullulanase** from K. aerogenes has similar active sites to those of alpha-amylases with the four conserved regions, as well as another substrate-binding site closer to the NH2-terminus. The plate assay method used for isolation of thermostable **variants** may be applicable to the generation of useful **variants** of other enzymes.

L9 ANSWER 10 OF 21 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
ACCESSION NUMBER: 1995:123729 BIOSIS
DOCUMENT NUMBER: PREV199598138029
TITLE: The genetic manipulation of the yeast Saccharomyces cerevisiae with the aim of converting polysaccharide-rich agricultural crops and industrial waste to single-cell protein and fuel ethanol.
AUTHOR(S): Pretorius, I. S.
CORPORATE SOURCE: Dep. Mikrobiol., Inst. Biotegnol., Univ. van Stellenbosch, Stellenbosch 7600 South Africa
SOURCE: Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie,
(1994) Vol. 13, No. 3, pp. 66-80.
ISSN: 0254-3486.
DOCUMENT TYPE: General Review
LANGUAGE: Afrikaans
SUMMARY LANGUAGE: Afrikaans; English
AB The world's problem with overpopulation and environmental pollution has created an urgent demand for alternative protein and energy sources. One way of addressing these burning issues is to produce single-cell protein (for food and animal feed supplements) and fuel ethanol from polysaccharide-rich agricultural crops and industrial waste by using baker's yeast. Owing to the absence of certain depolymerising enzymes, the yeast Saccharomyces cerevisiae is unable to utilise the vast reserves of energy sources present in starch, pectin, cellulose and hemicellulose. Enzymes such as amylases, pectinases, cellulases and hemicellulases are required for the release of fermentable sugars from these polysaccharides. For the complete conversion of starch to glucose one requires a liquefaction enzyme (alpha-amylase), a saccharifying enzyme (glucoamylase) and a debranching enzyme (**pullulanase**). Thus far we have cloned, manipulated and expressed the alpha-amylase gene (AMY1) from the Gram-positive **bacterium** **Bacillus** amyloliquefaciens, the glucoamylase gene (STA2) from S. cerevisiae var. diastaticus and the **pullulanase** gene (PUL1) from the Gram-negative **bacterium** **Klebsiella** **pneumoniae** in S. cerevisiae. To circumvent the expensive pretreatment (a cooking process) of starch in future industrial plants, we have also cloned the genes (RSA1 and RSG1) encoding raw starch-degrading amylases from the yeast Endomyces fibuliger and are currently endeavoring to incorporate these genes into the existing amylase cassette to be expressed in S. cerevisiae. The bioconversion of pectin is catalysed by pectinesterases and depolymerases. Some strains of S. cerevisiae produce pectinesterase and can convert pectin into pectate. A pectinase cassette comprising yeast expression/secretion systems that contain a pectate lyase gene (PEL5) from the plant pathogen Erwinia chrysanthemi, and the polygalacturonase gene (PEH1) from Erwinia carotovora was designed and successfully expressed in S. cerevisiae. The

most important enzymes involved in the degradation and utilisation of cellulose and hemicellulose can be divided into the following groups: endoglucanase (glucanohydrolase), exoglucanase (cellobiohydrolase), cellobiase (beta-glucosidase), beta-xylanase, beta-xylosidase and xylose isomerase. We have cloned, **modified** and expressed the endo-beta-1,4-glucanase gene (END1) from the rumen **bacterium** Butyrivibrio fibrisolvens, the exo- and endo-beta-1,3-glucanase genes (BGL1/EXG1 and BGL2/ENG2) from S. cerevisiae, the cellobiase and beta-glucosidase genes (BGL1 and BGL2) from E. fibuliger and the beta-xylanase genes (XYN2 and XYN3) from Trichoderma reesei and Aspergillus kawachii in S. cerevisiae. The cellobiohydrolase gene (CBH1) from the white rot fungus Phanerochaeta chrysosporium and the xylanase gene (XYN1) from the **bacterium** Ruminococcus flavefaciens are currently being prepared for expression in S. cerevisiae. At the same time, our laboratories are also seeking to clone and express the genes encoding beta-xylanase (XYN1), beta-xylosidase (XYL1) and xylose-isomerase (XYS1) from the fungi T. reesei, Aspergillus niger and Candida boidinii. Eventually we will endeavour to combine these amylase, pectinase, cellulase and hemicellulase cassettes onto an artificial minichromosome and introduce it into S. cerevisiae, thereby enabling baker's yeast to utilise these different polysaccharides.

L9 ANSWER 11 OF 21 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:553380 CAPLUS
DOCUMENT NUMBER: 119:153380
TITLE: Gene expressing in **Bacillus** licheniformis using especially .alpha.-amylase promoter **variant**
INVENTOR(S): Joergensen, Steen Troels; Joergensen, Per Linaa
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 63 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9310248	A1	19930527	WO 1992-DK337	19921113
W: FI, JP, KR				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE				
JP 07503363	T2	19950413	JP 1993-508898	19921113
EP 672154	A1	19950920	EP 1992-923721	19921113
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
FI 9402227	A	19940513	FI 1994-2227	19940513

PRIORITY APPLN. INFO.: WO 1991-DK344 19911114
WO 1992-DK337 19921113

AB Genes of anaerobic and/or thermophilic microorganisms are expressed in B. licheniformis from a promoter **variant** of .alpha.-amylase (I) gene of B. licheniformis. Plasmid pSJ1391 contg. fusion gene for I/CGTase (cyclodextrin glycosyl transferase) expressed from the promoter **variant** of I gene of B. licheniformis was constructed. The plasmid was transformed into an I-producing B. licheniformis for integration of the fusion gene by in vivo recombination. B. subtilis transformants contg. the fusion gene integrated into the chromosome was similarly prepd. The recombinant B. licheniformis and B. subtilis produced CGTase 200-275 and 17-21 arbitrary units, resp.

L9 ANSWER 12 OF 21 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 93:38575 SCISEARCH
THE GENUINE ARTICLE: KG621
TITLE: STABLE PERIPLASMIC SECRETION INTERMEDIATE IN THE GENERAL SECRETORY PATHWAY OF ESCHERICHIA-COLI

AUTHOR: POQUET I; FAUCHER D; PUGSLEY A P (Reprint)
CORPORATE SOURCE: I[redacted] PASTEUR, UNITE GENET MOLEC, C[redacted], URA 1149, 25 RUE DR ROUX, F-75724 PARIS 15, FRANCE; RHONE POULENC RORER, DEPT BIOTECHNOL, F-94403 VITRY, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: EMBO JOURNAL, (JAN 1993) Vol. 12, No. 1, pp. 271-278. ISSN: 0261-4189.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 43

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The secretion of the **Klebsiella** oxytoca cell surface lipoprotein **pullulanase** involves translocation across the cytoplasmic and outer membranes of the Gram-negative **bacterial** cell envelope. A **variant** of **pullulanase** was created by fusing the signal peptide-encoding 5' region of the Escherichia coli gene for periplasmic MalE protein to the 3' end of the pulA gene encoding almost the entire mature part of **pullulanase**. When produced in E.coli carrying the malE-pulA gene fusion on a high copy number plasmid and the complete set of genes specifically required for **pullulanase** secretion on a second plasmid, the hybrid protein differed from wild-type **pullulanase** as follows: (i) it was not fatty-acylated; (ii) it was apparently processed by LepB signal peptidase rather than by LspA lipoprotein signal peptidase; (iii) it was released into the periplasm and was only slowly transported across the outer membrane, and (iv) it was released directly into the medium rather than via the usual surface-anchored intermediate. The hybrid protein was secreted more rapidly when malE-pulA was expressed from a low copy number plasmid. The two steps in the secretion pathway could be totally uncoupled by expressing first the malE-pulA gene fusion and then the cognate secretion genes. These results show that fatty-acylation of wild-type PulA is not essential for secretion but may improve its efficiency when large amounts of the protein are produced, that the two steps in secretion can occur quite independently and that the periplasmic intermediate can persist for long periods under certain circumstances.

L9 ANSWER 13 OF 21 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 91:496254 SCISEARCH
THE GENUINE ARTICLE: GD635
TITLE: THE PROTEIN-SEQUENCE RESPONSIBLE FOR LIPOPROTEIN MEMBRANE LOCALIZATION IN ESCHERICHIA-COLI EXHIBITS REMARKABLE SPECIFICITY
AUTHOR: GENNITY J M; INOUYE M (Reprint)
CORPORATE SOURCE: UNIV MED & DENT NEW JERSEY, ROBERT WOOD JOHNSON MED SCH, DEPT BIOCHEM, PISCATAWAY, NJ, 08854
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1991) Vol. 266, No. 25, pp. 16458-16464.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 44

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Structural information defining an N-terminal sequence required for the membrane sorting of **bacterial** lipoproteins has been previously garnered through the study of a hybrid outer membrane (OM) lipo-beta-lactamase (LL) (Ghrayeb and Inouye (1984) J. Biol. Chem. 259, 463-467). Introduction of an aspartate as the second residue of mature LL (D2 **mutant**) causes an inner membrane (IM) localization of this protein (Yamaguchi, K., Yu, F., and Inouye, M. (1988) Cell 53, 423-432). Introduction of as aspartate at the third residue of mature LL (D3) causes

a weaker IM sorting signal and when present as the fourth residue (D4), normal OM sorting occurs. A positively charged residue at the second position (K2) has no effect on OM localization. Remarkably, glutamate substitution at either the second (E2) or third (E3) position does not interfere with OM sorting. Sorting of the **mutant** D2 LL can be partially suppressed by introduction of a positively charged histidine (D2H3) or lysine (D2K3) at residue 3 of the mature protein. These results indicate that both the negative charge of the aspartate residue and some structural feature not present in a glutamate residue are required for sorting to the IM. The suppression of IM localization of the D2H3 LL double **mutant** can be eliminated by growing Escherichia coli at pH 8.4 to reduce the histidine partial positive charge. This result supports the essentiality of a negative charge in IM localization and indicates that the committed step in lipoprotein sorting is made in a cellular compartment, the periplasm, at equilibrium with the external pH.

L9 ANSWER 14 OF 21 MEDLINE
ACCESSION NUMBER: 91126059 MEDLINE
DOCUMENT NUMBER: 91126059 PubMed ID: 1992458
TITLE: Cloned Erwinia chrysanthemi out genes enable Escherichia coli to selectively secrete a diverse family of heterologous proteins to its milieu.
AUTHOR: He S Y; Lindeberg M; Chatterjee A K; Collmer A
CORPORATE SOURCE: Department of Plant Pathology, Cornell University, Ithaca, NY 14853.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1991 Feb 1) 88 (3) 1079-83. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M37886; GENBANK-M80723; GENBANK-M80724; GENBANK-M81660; GENBANK-M81661; GENBANK-M81662; GENBANK-M81664; GENBANK-M81665; GENBANK-M81666; GENBANK-S68027
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910405
Last Updated on STN: 19970203
Entered Medline: 19910308

AB The out genes of the enterobacterial plant pathogen Erwinia chrysanthemi are responsible for the efficient extracellular secretion of multiple plant cell wall-degrading enzymes, including four isozymes of pectate lyase, exo-poly-alpha-D-galacturonosidase, pectin methylesterase, and cellulase. Out- **mutants** of Er. chrysanthemi are unable to export any of these proteins beyond the periplasm and are severely reduced in virulence. We have cloned out genes from Er. chrysanthemi in the stable, low-copy-number cosmid pCPP19 by complementing several transposon-induced mutations. The cloned out genes were clustered in a 12-kilobase chromosomal DNA region, complemented all existing out mutations in Er. chrysanthemi EC16, and enabled Escherichia coli strains to efficiently secrete the extracellular pectic enzymes produced from cloned Er. chrysanthemi genes, while retaining the periplasmic marker protein beta-lactamase. DNA sequencing of a 2.4-kilobase EcoRI fragment within the out cluster revealed four genes arranged colinearly and sharing substantial similarity with the **Klebsiella pneumoniae** genes pulH, pulI, pulJ, and pulK, which are necessary for **pullulanase** secretion. However, K. **pneumoniae** cells harboring the cloned Er. chrysanthemi pelE gene were unable to secrete the Erwinia pectate lyase. Furthermore, the Er. chrysanthemi Out system was unable to secrete an extracellular pectate lyase encoded by a gene from a closely related plant pathogen. Erwinia carotovora ssp. carotovora. The results suggest that these enterobacteria secrete polysaccharidases by a conserved mechanism whose protein-recognition capacities have diverged.

L9 ANSWER 15 OF 21 EMBASE COPYRIGHT 2001 ELSEVIER S[redacted] B.V.
ACCESSION NUMBER: 91[redacted]9794 EMBASE
DOCUMENT NUMBER: 1991049794
TITLE: Protein secretion in Pseudomonas aeruginosa: The xcpA gene encodes an integral inner membrane protein homologous to **Klebsiella pneumoniae** secretion function protein PulO.
AUTHOR: Bally M.; Ball G.; Badere A.; Lazdunski A.
CORPORATE SOURCE: Lab. de Chimie Bacterienne, Centre National, de la Recherche Scientifique, 31, Chemin Joseph Aiguier,13402 Marseille Cedex 9, France
SOURCE: Journal of Bacteriology, (1991) 173/2 (479-486). ISSN: 0021-9193 CODEN: JOBAAY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB xcp mutations have pleiotropic effects on the secretion of proteins in Pseudomonas aeruginosa PAO. The nucleotide sequence of a 1.2-kb DNA fragment that complements the xcp-1 mutation has been determined. Sequence analysis shows the xcpA gene product to be a 31.8-kDa polypeptide, with a highly hydrophobic character. This is consistent with a localization in the cytoplasmic membrane in P. aeruginosa, determined after specific expression of the xcpA gene under control of the T7.PHI.10 promoter. A very strong homology was found between XcpA and PulO, a membrane protein required for **pullulanase** secretion in **Klebsiella pneumoniae**. This suggests the existence of a signal sequence-dependent secretion process common to these two unrelated gram-negative **bacteria**.

L9 ANSWER 16 OF 21 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 91:571512 SCISEARCH
THE GENUINE ARTICLE: GK112
TITLE: CONSERVATION OF XCP GENES, INVOLVED IN THE 2-STEP PROTEIN SECRETION PROCESS, IN DIFFERENT PSEUDOMONAS SPECIES AND OTHER GRAM-NEGATIVE **BACTERIA**
AUTHOR: DEGROOT A (Reprint); FILLOUX A; TOMMASSEN J
CORPORATE SOURCE: STATE UNIV UTRECHT, DEPT MOLEC CELL BIOL, PADUALAAN 8, 3584 CH UTRECHT, NETHERLANDS (Reprint); STATE UNIV UTRECHT, INST MOLEC BIOL & MED BIOTECHNOL, 3584 CH UTRECHT, NETHERLANDS
COUNTRY OF AUTHOR: NETHERLANDS
SOURCE: MOLECULAR & GENERAL GENETICS, (1991) Vol. 229, No. 2, pp. 278-284.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 42

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The two-step protein secretion pathway in Pseudomonas aeruginosa is dependent on the xcp genes. We investigated whether a similar secretion mechanism is present in non-pathogenic Pseudomonas spp. and in other gram-negative **bacteria**. The plant growth stimulating Pseudomonas strains P. putida WCS358, P. fluorescens WCS374 and Pseudomonas B10 appeared to secrete proteins into the extracellular medium. Southern hybridization experiments showed the presence of xcp genes in these strains and also in other gram-negative **bacteria**, including Xanthomonas campestris. Complementation experiments showed that the xcp gene cluster of P. aeruginosa restored protein secretion in an X. campestris secretion **mutant**. The secretion gene cluster of X. campestris however, restored secretion capacity in P. aeruginosa mutants only to a low degree. Two heterologous proteins were not

secreted by P. fluorescens and P. aeruginosa. The results suggest the presence of a similar two-step protein secretion mechanism in different gram-negative **bacteria**, which however, is not always functional for heterologous proteins.

L9 ANSWER 17 OF 21 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 91285432 MEDLINE
DOCUMENT NUMBER: 91285432 PubMed ID: 1676385
TITLE: Characterisation of a Pseudomonas aeruginosa twitching motility gene and evidence for a specialised protein export system widespread in eubacteria.
AUTHOR: Whitchurch C B; Hobbs M; Livingston S P; Krishnapillai V; Mattick J S
CORPORATE SOURCE: Centre for Molecular Biology and Biotechnology, University of Queensland, Brisbane, Australia.
SOURCE: GENE, (1991 May 15) 101 (1) 33-44.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M55524
ENTRY MONTH: 199108
ENTRY DATE: Entered STN: 19910825
Last Updated on STN: 19950206
Entered Medline: 19910805

AB Type-4 fimbriae (pili) are associated with a phenomenon known as twitching motility, which appears to be involved with **bacterial** translocation across solid surfaces. Pseudomonas aeruginosa **mutants** which produce fimbriae, but which have lost the twitching motility function, display altered colony morphology and resistance to fimbrial-specific **bacteriophage**. We have used phenotypic complementation of such **mutants** to isolate a region of DNA involved in twitching motility. This region was physically mapped to a SpeI fragment around 20 min on the P. aeruginosa PAO chromosome, remote from the major fimbrial locus (around 75 min) where the structural subunit-encoding gene (fimA/pilA) and ancillary genes required for fimbrial assembly (pilB, C and D) are found. A gene, pilT, within the twitching motility region is predicted to encode a 344-amino acid protein which has strong homology to a variety of other **bacterial** proteins. These include the P. aeruginosa PilB protein, the ComG ORF-1 protein from the **Bacillus** subtilis comG operon (necessary for competence), the PulE protein from the **Klebsiella** oxytoca (formerly K. **pneumoniae**) pulC-O operon (involved in **pullulanase** export), and the VirB-11 protein from the virB operon (involved in virulence) which is located on the Agrobacterium tumefaciens Ti plasmid. We have also identified other sets of homologies between P. aeruginosa fimbrial assembly (Pil) proteins and B. subtilis Com and K. oxytoca Pul proteins, which suggest that these are all related members of a specialised protein export pathway which is widespread in the eubacteria.

L9 ANSWER 18 OF 21 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 91:5879 SCISEARCH
THE GEN'INE ARTICLE: EN921
TITLE: PROTEIN SECRETION IN GRAM-NEGATIVE **BACTERIA** - TRANSPORT ACROSS THE OUTER-MEMBRANE INVOLVES COMMON MECHANISMS IN DIFFERENT **BACTERIA**
AUTHOR: FILLOUX A (Reprint); BALLY M; BALL G; AKRIM M; TOMMASSEN J; LAZDUNSKI A
CORPORATE SOURCE: STATE UNIV UTRECHT, DEPT MOLEC CELL BIOL, PADUALAAN 8, 3584 CH UTRECHT, NETHERLANDS (Reprint); CNRS, CHIM BACTERIENNE LAB, F-13402 MARSEILLE 9, FRANCE
COUNTRY OF AUTHOR: NETHERLANDS; FRANCE

SOURCE: EMBO JOURNAL, (1990) Vol. 9, No. 13, pp. 4323-4329.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 43

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The xcp genes are required for protein secretion by Pseudomonas aeruginosa. They are involved in the second step of the process, i.e. the translocation across the outer membrane, after the exoproteins have reached the periplasm in a signal peptide dependent fashion. The nucleotide sequence of a 2.5 kb DNA fragment containing xcp genes showed at least two complete open reading frames, potentially encoding proteins with molecular weights of 41 and 19 kd. Products with these apparent molecular weights were identified after expression of the DNA fragment in vitro and in vivo. Subcloning and complementation experiments showed that both proteins are required for secretion. The two products are located in the inner membrane and share highly significant homologies with the PulL and PulM proteins which are required for the specific secretion of **pullulanase** in **Klebsiella** pnuemoniae. These homologies reveal the existence of a common mechanism for protein secretion in Pseudomonas aeruginosa and **Klebsiella pneumoniae**.

L9 ANSWER 19 OF 21 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 90132554 MEDLINE
DOCUMENT NUMBER: 90132554 PubMed ID: 2693596
TITLE: A new regulatory locus of the maltose regulon in **Klebsiella pneumoniae** strain K21 identified by the study of **pullulanase** secretion **mutants**.
AUTHOR: Kornacker M G; Boyd A; Pugsley A P; Plastow G S
CORPORATE SOURCE: Leicester Biocentre, University of Leicester, UK.
SOURCE: JOURNAL OF GENERAL MICROBIOLOGY, (1989 Feb) 135 (Pt 2) 397-408.
Journal code: I87; 0375371. ISSN: 0022-1287.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199003
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19900328
Entered Medline: 19900315

AB This study has shown that **Klebsiella pneumoniae** strain K21 differs from the previously characterized and closely related K. pneumoniae strain PAP996 in that expression of the **pullulanase** gene (pulA) and other genes of the maltose regulon is partially independent of exogenous inducer (maltose/maltotriose). Mutants of strain K21 which are defective in **pullulanase** synthesis and/or secretion were isolated following Tn10 mutagenesis. Three phenotypic classes of **mutants** were identified. Class I mutants were defective in the surface localization and secretion of **pullulanase**. Class II **mutants** did not secrete detectable levels of **pullulanase** but were able to export **pullulanase** to the cell surface. Class II **mutants** also expressed **pullulanase** and other maltose-regulated genes at markedly lower levels than those found in the parent strain under non-inducing conditions. The single class III **mutant** was intermediate between K21 and class I **mutants**; most of the cell-associated **pullulanase** was localized at the cell surface whilst a significant amount was secreted into the medium. Mapping indicated that all but three of the Tn10 insertions were adjacent to, and at either side of, pulA. One class II **mutant** carried a Tn10

insertion in or close to malT whereas in the remaining class II mutants the insertions were located at least 4 kb upstream of pulA in a region which may define a new regulatory locus of the maltose operon.

L9 ANSWER 20 OF 21 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 86223769 MEDLINE
DOCUMENT NUMBER: 86223769 PubMed ID: 3519575
TITLE: Extracellular **pullulanase** of **Klebsiella pneumoniae** is a lipoprotein.
AUTHOR: Pugsley A P; Chapon C; Schwartz M
SOURCE: JOURNAL OF BACTERIOLOGY, (1986 Jun) 166 (3) 1083-8.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198607
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19860717

AB **Pullulanase** is a starch-debranching enzyme produced by the gram-negative **bacterium Klebsiella pneumoniae**. In this organism, the enzyme is first exported to the outer membrane and is subsequently released into the growth medium. Evidence reported here indicates that **pullulanase** is a lipoprotein. It is apparently synthesized as a precursor with a 19-residue-long signal sequence and modified by the covalent attachment of palmitate to the cysteine residue which becomes the amino terminus after cleavage of the signal sequence. In this respect, **pullulanase** is similar to some penicillinases produced by gram-positive **bacteria** which are initially exported to the cell surface and subsequently released into the medium. However, **pullulanase** and the penicillinases differ in one important aspect, namely, that the extracellular **pullulanase** still carries the covalently attached fatty acyls, whereas extracellular penicillinases lack the **modified** amino-terminal cysteine together with a limited number of other residues from the amino terminus.

L9 ANSWER 21 OF 21 MEDLINE
ACCESSION NUMBER: 86033620 MEDLINE
DOCUMENT NUMBER: 86033620 PubMed ID: 3902791
TITLE: Characterization and expression of the structural gene for **pullulanase**, a maltose-inducible secreted protein of **Klebsiella pneumoniae**.
AUTHOR: Michaelis S; Chapon C; D'Enfert C; Pugsley A P; Schwartz M
SOURCE: JOURNAL OF BACTERIOLOGY, (1985 Nov) 164 (2) 633-8.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198512
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19851219

AB Some strains of **Klebsiella** pneumonia secrete **pullulanase**, a debranching enzyme which produces linear molecules (maltodextrins, amylose) from amylopectin and glycogen. pulA, the structural gene for **pullulanase**, was introduced into Escherichia coli, either on a multiple-copy-number plasmid or as a single copy in the chromosome. When in E. coli, pulA was controlled by malT, the positive regulatory gene of the maltose regulon. Indeed, pulA expression was undetectable in a malT-negative **mutant** and constitutive in a malTc strain. Furthermore, the plasmid carrying pulA titrated the MalT protein. When produced in E. coli, **pullulanase** was not localized in the same

way as in K. **pneumoniae**. In the latter case it was first exported to the outer membrane, with which it remained loosely associated, and was then released into the growth medium. In E. coli the enzyme was distributed both in the inner and the outer membranes and was never released into the growth medium.

=> d l10 ibib ab 1-4

L10 ANSWER 1 OF 4 MEDLINE
ACCESSION NUMBER: 95221318 MEDLINE
DOCUMENT NUMBER: 95221318 PubMed ID: 7706211
TITLE: Random mutagenesis of **pullulanase** from **Klebsiella** aerogenes for studies of the structure and function of the enzyme.
AUTHOR: Yamashita M; Kinoshita T; Ihara M; Mikawa T; Murooka Y
CORPORATE SOURCE: Department of Fermentation Technology, Faculty of Engineering, Hiroshima University.
SOURCE: JOURNAL OF BIOCHEMISTRY, (1994 Dec) 116 (6) 1233-40.
Journal code: HIF; 0376600. ISSN: 0021-924X.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950518
Last Updated on STN: 19970203
Entered Medline: 19950511

QP561.J6
N/A

AB To study the structure and function of **pullulanase** from **Klebsiella** aerogenes, a method involving random mutagenesis of the entire gene for **pullulanase** was used. Out of 50,000 **clones** screened at high temperature, seven genes for mutant proteins were identified by DNA sequencing. The amino acid substitutions in the seven **mutant** proteins were clustered on the NH2-terminal side of the four conserved regions found in alpha-amylases. These **mutant pullulanases** were classified into two types: those whose catalytic activity was altered and those whose thermal stability was increased. The results presented here and in previous reports suggest that **pullulanase** from K. aerogenes has similar active sites to those of alpha-amylases with the four conserved regions, as well as another substrate-binding site closer to the NH2-terminus. The plate assay method used for isolation of thermostable **variants** may be applicable to the generation of useful **variants** of other enzymes.

L10 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:123729 BIOSIS
DOCUMENT NUMBER: PREV199598138029
TITLE: The genetic manipulation of the yeast Saccharomyces cerevisiae with the aim of converting polysaccharide-rich agricultural crops and industrial waste to single-cell protein and fuel ethanol.
AUTHOR(S): Pretorius, I. S.
CORPORATE SOURCE: Dep. Mikrobiol., Inst. Biotegnol., Univ. van Stellenbosch, Stellenbosch 7600 South Africa
SOURCE: Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie,
(1994) Vol. 13, No. 3, pp. 66-80.
ISSN: 0254-3486.
DOCUMENT TYPE: General Review
LANGUAGE: Afrikaans
SUMMARY LANGUAGE: Afrikaans; English
AB The world's problem with overpopulation and environmental pollution has created an urgent demand for alternative protein and energy sources. One way of addressing these burning issues is to produce single-cell protein

(for food and animal feed supplements) and fuel ethanol from polysaccharide-rich agricultural crops and industrial waste by using baker's yeast. Owing to the absence of certain depolymerising enzymes, the yeast Saccharomyces cerevisiae is unable to utilise the vast reserves of energy sources present in starch, pectin, cellulose and hemicellulose. Enzymes such as amylases, pectinases, cellulases and hemicellulases are required for the release of fermentable sugars from these polysaccharides. For the complete conversion of starch to glucose one requires a liquefaction enzyme (alpha-amylase), a saccharifying enzyme (glucoamylase) and a debranching enzyme (**pullulanase**). Thus far we have cloned, manipulated and expressed the alpha-amylase gene (AMY1) from the Gram-positive **bacterium Bacillus** amyloliquefaciens, the glucoamylase gene (STA2) from S. cerevisiae var. diastaticus and the **pullulanase** gene (PUL1) from the Gram-negative **bacterium Klebsiella pneumoniae** in S. cerevisiae. To circumvent the expensive pretreatment (a cooking process) of starch in future industrial plants, we have also cloned the genes (RSA1 and RSG1) encoding raw starch-degrading amylases from the yeast Endomyces fibuliger and are currently endeavoring to incorporate these genes into the existing amylase cassette to be expressed in S. cerevisiae. The bioconversion of pectin is catalysed by pectinesterases and depolymerases. Some strains of S. cerevisiae produce pectinesterase and can convert pectin into pectate. A pectinase cassette comprising yeast expression/secretion systems that contain a pectate lyase gene (PEL5) from the plant pathogen Erwinia chrysanthemi, and the polygalacturonase gene (PEH1) from Erwinia carotovora was designed and successfully expressed in S. cerevisiae. The most important enzymes involved in the degradation and utilisation of cellulose and hemicellulose can be divided into the following groups: endoglucanase (glucanohydrolase), exoglucanase (cellobiohydrolase), cellobiase (beta-glucosidase), beta-xylanase, beta-xylosidase and xylose isomerase. We have cloned, **modified** and expressed the endo-beta-1,4-glucanase gene (END1) from the rumen **bacterium** Butyrivibrio fibrisolvens, the exo- and endo-beta-1,3-glucanase genes (BGL1/EXG1 and BGL2/ENG2) from S. cerevisiae, the cellobiase and beta-glucosidase genes (BGL1 and BGL2) from E. fibuliger and the beta-xylanase genes (XYN2 and XYN3) from Trichoderma reesei and Aspergillus kawachii in S. cerevisiae. The cellobiohydrolase gene (CBH1) from the white rot fungus Phanerochaeta chrysosporium and the xylanase gene (XYN1) from the **bacterium** Ruminococcus flavefaciens are currently being prepared for expression in S. cerevisiae. At the same time, our laboratories are also seeking to **clone** and express the genes encoding beta-xylanase (XYN1), beta-xylosidase (XYL1) and xylose-isomerase (XYS1) from the fungi T. reesei, Aspergillus niger and Candida boidinii. Eventually we will endeavour to combine these amylase, pectinase, cellulase and hemicellulase cassettes onto an artificial minichromosome and introduce it into S. cerevisiae, thereby enabling baker's yeast to utilise these different polysaccharides.

L10 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:34954 CAPLUS
DOCUMENT NUMBER: 132:90065
TITLE: Genetic engineering of starch-debranching enzymes for improved thermostability and specificity
INVENTOR(S): Bisgard-Frantzen, Henrik; Svendsen, Allan
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000001796	A2	20000113	WO 1999-DK381	19990702
WO 2000001796	A3	20000309		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9948971	A1	20000124	AU 1999-48971	19990702
EP 1092014	A2	20010418	EP 1999-932675	19990702

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRIORITY APPLN. INFO.:	DK 1998-868	A 19980702
	WO 1999-DK381	W 19990702

AB The invention relates to a genetically engineered **variant** of a parent starch-debranching enzyme, i.e. a **pullulanase** or an isoamylase, the enzyme **variant** having an improved thermostability at a pH in the range of 4-6 compared to the parent enzyme and/or an increased activity towards amylopectin and/or glycogen compared to the parent enzyme. Methods for producing such starch-debranching enzyme **variants** with improved thermostability and/or altered substrate specificity are provided. Alignment of **pullulanases** of **Bacillus** acidopullulyticus and **Bacillus** deramificans, and of isoamylases of Rhodothermus marinus and Pseudomonas amyloderamosa, identified specific loop regions and amino acid residues appropriate for substitution with thermostability-conferring residues. The **modified** enzymes should yield improved conversion of starch to one or more sugars.

L10 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:553380 CAPLUS
DOCUMENT NUMBER: 119:153380
TITLE: Gene expressing in **Bacillus** licheniformis using especially .alpha.-amylase promoter **variant**
INVENTOR(S): Joergensen, Steen Troels; Joergensen, Per Linaa
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 63 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9310248	A1	19930527	WO 1992-DK337	19921113

W: FI, JP, KR

RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE

JP 07503363	T2	19950413	JP 1993-508898	19921113
EP 617154	A1	19950920	EP 1992-923721	19921113

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE

FI 9402227	A	19940513	FI 1994-2227	19940513

PRIORITY APPLN. INFO.:	WO 1991-DK344	19911114
	WO 1992-DK337	19921113

AB Genes of anaerobic and/or thermophilic microorganisms are expressed in B. licheniformis from a promoter **variant** of .alpha.-amylase (I) gene of B. licheniformis. Plasmid pSJ1391 contg. fusion gene for I/CGTase (cyclodextrin glycosyl transferase) expressed from the promoter

variant of I gene of B. licheniformis was constructed. The plasmid was transformed into an I-producing B. licheniformis for integration of the fusion gene by in vivo recombination. B. subtilis transformants contg. the fusion gene integrated into the chromosome was similarly prepd. The recombinant B. licheniformis and B. subtilis produced CGTase 200-275 and 17-21 arbitrary units, resp.

=> d l5 ibib ab 1-16

L5 ANSWER 1 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 2001:83642 SCISEARCH
THE GENUINE ARTICLE: 393FX
TITLE: Exchange of Xcp (Gsp) secretion machineries between Pseudomonas aeruginosa and Pseudomonas alcaligenes: Species specificity unrelated to substrate recognition
AUTHOR: De Groot A; Koster M; Gerard-Vincent M; Gerritse G; Lazdunski A; Tommassen J; Filloux A (Reprint)
CORPORATE SOURCE: IBSM, Lab Ingn Syst Macromol, CNRS, UPR 9027, 31 Chemin Joseph Aiguier, F-13402 Marseille 20, France (Reprint); IBSM, Lab Ingn Syst Macromol, CNRS, UPR 9027, F-13402 Marseille 20, France; Univ Utrecht, Inst Biomembranes, NL-3584 CH Utrecht, Netherlands; Genencor Int BV, NL-2300 AE Leiden, Netherlands
COUNTRY OF AUTHOR: France; Netherlands
SOURCE: JOURNAL OF BACTERIOLOGY, (FEB 2001) Vol. 183, No. 3, pp. 959-967.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 60
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pseudomonas aeruginosa and Pseudomonas alcaligenes are gram-negative bacteria that secrete proteins using the type II or general secretory pathway, which requires at least 12 xcp gene products (XcpA and XcpP to -Z), Despite strong conservation of this secretion pathway, gram-negative bacteria usually cannot secrete exoproteins from other species. Based on results obtained with Erwinia, it has been proposed that the XcpP and/or XcpQ homologs determine this secretion specificity (M. Linderberg, G. P. Salmond, and A. Collmer, Mel. Microbiol. 20:175-190, 1996). In the present study, we report that XcpP and XcpQ of P. alcaligenes could not substitute for their respective P. aeruginosa counterparts. However, these complementation failures could not be correlated to species specific recognition of exoproteins, since these bacteria could secrete exoproteins of each other. Moreover, when P. alcaligenes xcpP and xcpQ were expressed simultaneously in a P. aeruginosa xcpPQ **deletion mutant** , complementation was observed, albeit only on agar plates and not in liquid cultures. After growth in liquid culture the heat-stable P. alcaligenes XcpQ multimers were not detected, whereas monomers were clearly visible. Together, our results indicate that the assembly of a functional Xcp machinery requires species-specific interactions between XcpP and XcpQ and between XcpP or XcpQ and another, as yet uncharacterized component(s).

L5 ANSWER 2 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 2001:39242 SCISEARCH
THE GENUINE ARTICLE: 387KJ
TITLE: Involvement of the XpsN protein in formation of the XpsL-XpsM complex in Xanthomonas campestris pv. campestris

type II secretion apparatus

AUTHOR: Lee H M; Tyan S W; Leu W M; Chen L Chen D C; Hu N T (Reprint)

CORPORATE SOURCE: Natl Chung Hsing Univ, Grad Inst Biol Chem, Taichung 40227, Taiwan (Reprint); Natl Chung Hsing Univ, Grad Inst Agr Biotechnol, Taichung 40227, Taiwan; Natl Chung Hsing Univ, Grad Inst Vet Microbiol, Taichung 40227, Taiwan; Natl Chung Hsing Univ, Agr Biotechnol Labs, Taichung 40227, Taiwan; Chung Shan Med & Dent Coll, Grad Inst Biochem, Taichung, Taiwan

COUNTRY OF AUTHOR: Taiwan

SOURCE: JOURNAL OF BACTERIOLOGY, (JAN 2001) Vol. 183, No. 2, pp. 528-535.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0021-9193.

DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 43

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The xps gene cluster is required for the second step of type II protein secretion in Xanthomonas campestris pv, campestris. **Deletion** of the entire gene cluster caused accumulation of secreted proteins in the periplasm. By analyzing protein abundance in the chromosomal mutant strains, we observed mutual dependence for normal steady-state levels between the XpsL and the XpsM proteins. The XpsL protein was undetectable in total lysate prepared from the xpsM mutant strain, and vice versa. Introduction of the wild-type xpsM gene carried on a plasmid into the xpsM **mutant** strain was sufficient for reappearance of the XpsL protein, and vice versa. Moreover, both XpsL and XpsM proteins were undetectable in the xpsN **mutant** strain. They were recovered either by reintroducing the wild-type xpsN gene or by introducing extra copies of wild-type xpsL or xpsM individually. Overproduction of wild-type XpsL and -M proteins simultaneously, but not separately, in the wild-type strain of X, campestris pv, campestris caused inhibition of secretion. Complementation of an xpsL or xpsM **mutant** strain with a plasmid-borne wild-type gene was inhibited by coexpression of XpsL and XpsM. The presence of the xpsN gene on the plasmid along with the xpsL and the xpsM genes caused more severe inhibition in both cases. Furthermore, complementation of the xpsM mutant strain was also inhibited. In both the wild-type strain and a strain with the xps gene cluster deleted (XC17433), carrying pLMN, which encodes all three proteins, each protein coprecipitated with the other two upon immunoprecipitation. Expression of pairwise combinations of the three proteins in XC17433 revealed that the XpsL-XpsM and XpsM-XpsN pairs still coprecipitated, whereas the XpsL-XpsN pair no longer coprecipitated.

L5 ANSWER 1 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)

ACCESSION NUMBER: 2000:185297 SCISEARCH
THE GEN. ARTICLE: 288VK

TITLE: Association of the cytoplasmic membrane protein XpsN with the outer membrane protein XpsD in the type II protein secretion apparatus of Xanthomonas campestris pv. campestris

AUTHOR: Lee H M; Wang K C; Liu Y L; Yew H Y; Chen L Y; Leu W M; Chen D C H; Hu N T (Reprint)

CORPORATE SOURCE: NATL CHUNGHSING UNIV, GRAD INST BIOL CHEM, 250 KUO KUANG RD, TAICHUNG 40227, TAIWAN (Reprint); NATL CHUNGHSING UNIV, GRAD INST BIOL CHEM, TAICHUNG 40227, TAIWAN; NATL CHUNGHSING UNIV, GRAD INST AGR BIOTECHNOL, TAICHUNG 40227, TAIWAN; NATL CHUNGHSING UNIV, GRAD INST MOL BIOL, TAICHUNG

40227, TAIWAN; NATL CHUNGHSING UNIV, GRAD INST BOT, TAICHUNG 40227, TAIWAN; NATL CHUNGHSING UNIV, GRAD INST VET MICROBIOL, TAICHUNG 40227, TAIWAN; NATL CHUNGHSING UNIV, AGR BIOTECHNOL LABS, TAICHUNG 40227, TAIWAN; CHUNG SHAN MED & DENT COLL, GRAD INST BIOCHEM, TAICHUNG, TAIWAN
COUNTRY OF AUTHOR: TAIWAN
SOURCE: JOURNAL OF BACTERIOLOGY, (MAR 2000) Vol. 182, No. 6, pp. 1549-1557.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 59

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An xps gene cluster composed of 11 open reading frames is required for the type II protein secretion in Xanthomonas campestris pv. campestris. Immediately upstream of the xpsD gene, which encodes an outer membrane protein that serves as the secretion channel by forming multimers, there exists an open reading frame (previously designated ORF2) that could encode a protein of 261 amino acid residues. Its N-terminal hydrophobic region is a likely membrane-anchoring sequence. Antibody raised against this protein could detect in the wild-type strain of X. campestris pv. campestris a protein band with an apparent molecular mass of 36 kDa by Western blotting. Its aberrant slow migration in sodium dodecyl sulfate-polyacrylamide gels might be due to its high proline content. We designated this protein XpsN. By constructing a **mutant** strain with an in-frame **deletion** of the chromosomal xpsN gene, we demonstrated that it is required for the secretion of extracellular enzyme by X. campestris pv. campestris. Subcellular fractionation studies indicated that the XpsN protein was tightly associated with the membrane. Sucrose gradient sedimentation followed by immunoblot analysis revealed that it primarily appeared in the cytoplasmic membrane fractions. Immune precipitation experiments indicated that the XpsN protein was coprecipitated with the XpsD protein. In addition, the XpsN protein was coeluted with the (His)(6)-tagged XpsD protein from the metal affinity chromatography column. All observations suggested that the XpsN protein forms a stable complex with the XpsD protein. In addition, immune precipitation analysis of the XpsN protein with various truncated XpsD proteins revealed that the C-terminal region of the XpsD protein between residues 650 and 759 was likely to be involved in complex formation between the two.

L5 ANSWER 16 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:577030 CAPLUS
DOCUMENT NUMBER: 131:196365
TITLE: N-terminal-truncated analogs of bacterial **pullulanases** retaining normal enzymic activity
INVENTOR(S): Miller, Brian S.; Shetty, Jayarama K.
PATENT ASSIGNEE(S): Genencor International, Inc., USA
SOURCE: PCT Int. Appl., 49 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9946394	A2	19990910	WO 1999-US4627	19990303
WO 9946394	A3	19991118		

DESIGNATED STATES: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,

MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI,
SK, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY,
KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK,
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9929301	A1	19990920	AU 1999-29801	19990303
BR 9908422	A	20001031	BR 1999-8422	19990303
EP 1060263	A2	20001220	EP 1999-911068	19990303

R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE, FI

PRIORITY APPLN. INFO.: US 1998-34630 A 19980304
WO 1999-US4627 W 19990303

AB **Pullulanases** from Bacillus and Klebsiella that retain normal 1,6-.alpha.-glycosidase activity despite having truncations of up to 300 amino acids from the N-terminal domain, optionally with further amino acid substitutions, and that may be useful in the starch industry are described. The present invention provides methods for producing the **modified pullulanase**, enzymic compns. comprising the **modified pullulanase**, and methods for the saccharification of starch comprising the use of the enzymic compns. Expression of the Bacillus deramificans **pullulanase** gene in B. licheniformis hosts lacking the Carlsberg subtilisin and endopeptidase GluC resulted in the appearance of a series of N-terminal deletions of the **pullulanase**. Saccharification of starch with mixts. of glucoamylase (20%) and the **pullulanases** (80%) led to the saccharification of the starch without the formation of disaccharides.

L5 ANSWER 5 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:48790 CAPLUS
DOCUMENT NUMBER: 130:106943
TITLE: **Variants** of Humicola family 6 endo-1,4-.beta.-glucanases CelA and CelB and their use in cleaning compositions
INVENTOR(S): Lund, Henrik; Nielsen, Jack Bech; Schulein, Martin; Damgaard, Bo; Andersen, Kim Vilbour
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 271 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9901541	A1	19990114	WO 1998-DK299	19980702

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9879088	A1	19990125	AU 1998-79088	19980702
EP 1002061	A1	20000524	EP 1998-929249	19980702

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI

PRIORITY APPLN. INFO.: DK 1997-813 19970704
WO 1998-DK299 19980702

AB Cleaning compns. are provided comprising one or more enzymes having cellulolytic activity wherein .gtoreq.25% of the total wt. of cellulolytic active protein derives from the presence of a Humicola

endo-1,4-.beta.-glucanase or Humicola-like cellulase of the glycolsyl hydrolase family [redacted] Thus, gene encoding two endo-.beta.-1,4-glucanases (cellulases CelA and CelB) were cloned from Humicola insolens. The 3-dimensional structure of the catalytic core domain of the 2 cellulases were solved by x-ray crystallog. methods. Amino acid sequence alignments with other known cellulases and mol. modeling allowed the identification of residues in the binding cleft of the catalytic core domain, its encompassing loop regions, and on the surface of the 3-dimensional structure. Mutagenesis allowed trimming of the binding cleft loops to increase activity. The CelB enzyme was also stabilized against denaturation by anionic tensides by mutation/**deletion** of surface exposed residues towards more neg. charged residue(s). The achieve improved performance of the enzyme in color clarification, a linker and cellulose-binding domain are attached to the catalytic core domain to achieve a hybrid enzyme. Addnl. **variants** were constructed. Addnl. **variants** were constructed (e.g. in positions 20, 56, 94, 96, 103, 182, 183 and 318) with altered pH activity, catalytic properties, and improved detergent compatibility.

REFERENCE COUNT: 11
REFERENCE(S): (2) Biomolecular Research Institute Ltd; WO 9502042 A1
1995 CAPLUS
(4) Novo Industri AS; WO 8909259 A1 1989 CAPLUS
(5) Novo Nordisk AS; WO 9407998 A1 1994 CAPLUS
(6) Novo Nordisk AS; WO 9524471 A1 1995 CAPLUS
(7) Novo Nordisk AS; WO 9623874 A1 1996 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1999:966278 SCISEARCH
THE GENUINE ARTICLE: 264QQ
TITLE: Testing the '+2 rule' for lipoprotein sorting in the Escherichia coli cell envelope with a new genetic selection
AUTHOR: Seydel A; Gounon P; Pugsley A P (Reprint)
CORPORATE SOURCE: INST PASTEUR, CNRS, URA 1773, UNITE GENET MOL, 25 RUE DR ROUX, F-75724 PARIS 15, FRANCE (Reprint); INST PASTEUR, CNRS, URA 1773, UNITE GENET MOL, F-75724 PARIS 15, FRANCE; INST PASTEUR, STN MICROSCOPIE ELECT, F-75724 PARIS 15, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: MOLECULAR MICROBIOLOGY, (14 DEC 1999) Vol. 34, No. 4, pp. 810-821.
Publisher: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND.
ISSN: 0950-382X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 42

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report a novel strategy for selecting mutations that mislocalize lipoproteins within the Escherichia coli cell envelope and describe the mutants obtained. A strain carrying a **deletion** of the chromosomal malE gene, coding for the periplasmic maltose-binding protein (MalE), cannot use maltose unless a wild-type copy of malE is present in trans. Replacement of the natural signal peptide of preMalE by the signal peptide and the first four amino acids of a cytoplasmic membrane-anchored lipoprotein resulted in N-terminal fatty acylation of MalE (lipoMalE) and anchoring to the periplasmic face of the cytoplasmic membrane, where it could still function. When the aspartate at position +2 of this protein was replaced by a serine, lipoMalE was sorted to the outer membrane, where it could not function. Chemical mutagenesis followed by selection for

maltose-using **mutants** resulted in the identification of two classes of mutation. The single class I **mutant** carried a plasmid-borne mutation that replaced the serine at position +2 by phenylalanine. Systematic substitutions of the amino acid at position +2 revealed that, besides phenylalanine, tryptophan, tyrosine, glycine and proline could all replace classical cytoplasmic membrane lipoprotein sorting signal (aspartate +2). Analysis of known and putative lipoproteins encoded by the E. coli K-12 genome indicated that these amino acids are rarely found at position +2. In the class II **mutants**, a chromosomal mutation caused small and variable amounts of lipoMalE to remain associated with the cytoplasmic membrane. Similar amounts of another, endogenous outer membrane lipoprotein, NlpD, were also present in the cytoplasmic membrane in these **mutants**, indicating a minor, general defect in the sorting of outer membrane lipoproteins. Four representative class II **mutants** analysed were shown not to carry mutations in the lolA or lolB genes, known to be involved in the sorting of lipoproteins to the outer membrane.

L5 ANSWER 7 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:794818 CAPLUS
DOCUMENT NUMBER: 130:106926
TITLE: **Pullulanase mutants** of Bacillus strain KSM-AP1378 for preparation of detergents and starch-saccharifying agents
INVENTOR(S): Sumitomo, Nobuyuki; Hatada, Yuji; Ichimura, Takashi; Saito, Kazuhiro; Kawai, Shuji; Ito, Susumu
PATENT ASSIGNEE(S): Kao Corp., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 19 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 10327768	A2	19981215	JP 1997-141596	19970530

AB Prepn. of **mutants** of **pullulanase** of Bacillus strain KSM-AP1378 by **deletion** or substitution mutation at 443-Met and/or 557-Ala to improved their resistance to oxidizing agents; and use of the **mutants** for the prepn. of detergents and starch-saccharifying agents are described. The **pullulanase** is derived from the domain 1023-Met.apprx.1820-Asp of the 1938-amino-acid amylopullulanase of Bacillus strain KSM-AP1378. Prepn. of single **mutants** M443A, M443E, M443I, M443L, M443N, M443R, M443S, and M443V; prepn. of double **mutants** such as M443L/A557C; their stability in the presence of H2O2; and the washing ability of a detergent compn. contg. them were also shown. Also claimed are the detergent and saccharifying agents contg. the **pullulanase mutants** and other enzymes such as .alpha.-amylase, glucoamylase, etc.

L5 ANSWER 8 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1998:14683 SCISEARCH
THE GENUINE ARTICLE: YL836
TITLE: The XcpR protein of Pseudomonas aeruginosa dimerizes via its N-terminus
AUTHOR: Turner L R; Olson J W; Lory S (Reprint)
CORPORATE SOURCE: UNIV WASHINGTON, SCH MED, DEPT MICROBIOL, SEATTLE, WA 98195 (Reprint); UNIV WASHINGTON, SCH MED, DEPT MICROBIOL, SEATTLE, WA 98195
COUNTRY OF AUTHOR: USA
SOURCE: MOLECULAR MICROBIOLOGY, (DEC 1997) Vol. 26, No. 5, pp. 877-887.

Publisher: BLACKWELL SCIENCE LTD, OSNEY MEAD, OXFORD, OX ENGLAND OX2 0EL.
ISSN: 0950-382X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 57
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Extracellular protein secretion by the main terminal branch of the general secretory pathway in Pseudomonas aeruginosa requires a secretion machinery comprising the products of at least 12 genes, One of the components of this machinery, the XcpR protein, belongs to a large family of related proteins distinguished by the presence of a highly conserved nucleotide binding domain (Walker box A), The XcpR protein is essential for the process of extracellular secretion and amino acid substitutions within the Walker A sequence result in inactive XcpR, The same mutations exert a dominant negative effect on protein secretion when expressed in wild-type bacteria. Transdominance of XcpR **mutants** suggests that this protein is involved in interactions with other components of the secretion machinery or that it functions as a multimer. In this study, the amino-terminal portion of the cI repressor protein of phage lambda was used as a reporter of dimerization in Escherichia coli following fusion to full-length as well as a truncated form of XcpR. The cI-XcpR hybrid proteins were able to dimerize, as demonstrated by the immunity of bacteria expressing them to killing by lambda phage. The full-length XcpR as well as several **deletion mutants** of XcpR were able to disrupt the dimerization of the chimeric cI-XcpR protein. The disruption of cI-XcpR dimers using the **deletion mutants** of XcpR, combined with the analysis of their dominant negative effects on protein secretion, was used to map the minimal dimerization domain of XcpR, which is located within an 85 amino acid region in its N-terminal domain, Taken together, the data presented in this paper suggest that the XcpR protein dimerizes via its N-terminus and that this dimerization is essential for extracellular protein secretion.

L5 ANSWER 9 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 96:104621 SCISEARCH
THE GENUINE ARTICLE: TT488
TITLE: XPSD, AN OUTER-MEMBRANE PROTEIN REQUIRED FOR PROTEIN SECRETION BY XANTHOMONAS-CAMPESTRIS PV CAMPESTRIS, FORMS A MULTIMER
AUTHOR: CHEN L Y; CHEN D Y; MIAW J; HU N T (Reprint)
CORPORATE SOURCE: NATL CHUNGHSING UNIV, AGR BIOTECHNOL LABS, 250 KUO KUANG RD, TAICHUNG 40227, TAIWAN (Reprint); NATL CHUNGHSING UNIV, AGR BIOTECHNOL LABS, TAICHUNG 40227, TAIWAN; NATL CHUNGHSING UNIV, INST MOLEC BIOL, TAICHUNG 40227, TAIWAN; CHUNG SHAN MED & DENT COLL, INST BIOCHEM, TAICHUNG, TAIWAN
COUNTRY OF AUTHOR: TAIWAN
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (02 FEB 1996) Vol. 271, No. 5, pp. 2703-2708.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 39
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB XpsD is an outer membrane lipoprotein, required for the secretion of extracellular enzymes by Xanthomonas campestris pv. campestris. Our previous studies indicated that when the xpsD gene was interrupted by transposon Tn5, extracellular enzymes were accumulated in the periplasm (Hu, N.-T., Hung, M.-N., Chiou, S.-J., Tang, F., Chiang, D.-C., Huang, H.-Y. and Wu, C.-Y. (1992) J. Bacteriol. 174, 2679-2687). In this study,

we constructed a series of substitutions and **deletion** **mutant** xpsD genes to investigate the roles of NH2- and COOH-terminal halves of XpsD in protein secretory function. Among these secretion defective xpsD mutations, one group (encoded by pCD105, pYL4, pKdA6, and pKD2) caused secretion interference when co-expressed with wild type xpsD, but the other (encoded by pMH7, pKdPs, and pKDT) did not. Cross-linking studies and gel filtration chromatography analysis indicated that the wild type XpsD protein forms a multimer in its native state. Similar gel filtration analysis of xpsD **mutants** revealed positive correlations between multimer formation and secretion interfering properties exerted by the **mutant** XpsD proteins in the parental strain XC1701. Those **mutant** XpsD proteins (encoded by pCD105, pYL4, pKdA6, and pKD2) that caused secretion interference formed multimers that are similar to the wild type XpsD multimers and those (encoded by pMH7, pKdPs, and pKDT) that did not formed smaller ones. Furthermore, gel filtration and anion exchange chromatography analyses indicated that the wild type XpsD protein co-fractionated with XpsD(Delta 29-428) or XpsD(Delta 448-650) protein but not with XpsD(Delta 74-303) or XpsD(Delta 553-759) protein. We propose that the **mutant** XpsD(Delta 29-428) protein caused secretion interference primarily by forming mixed nonfunctional multimers with the wild type XpsD protein in XC1701(pCD105). The **mutant** XpsD(Delta 74-303) did so by competing for unknown factor(s) in XC1701(pYL4).

L5 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:372926 CAPLUS
DOCUMENT NUMBER: 122:153390
TITLE: Bacillus xylanase and gene, expression vectors for the xylanase and other proteins, expression hosts therefor, and use of xylanase in pulp bleaching
INVENTOR(S): De, Buyl Eric; Lahaya, Andree; Ledoux, Pierre; Amory, Antoine; Detroz, Rene; Andre, Christophe; Vetter, Roman
PATENT ASSIGNEE(S): Solvay et Cie., Belg.
SOURCE: Eur. Pat. Appl., 78 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 634491	A1	19950118	EP 1994-202002	19940711
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, PT, SE				
GB 2279957	A1	19950118	GB 1993-14780	19930715
GB 2279957	B2	19980218		
AU 9467432	A1	19950127	AU 1994-67432	19940713
AU 687828	B2	19980305		
CA 2128050	AA	19950116	CA 1994-2128050	19940714
NO 9402652	A	19950116	NO 1994-2652	19940714
FI 9403389	A	19950116	FI 1994-3389	19940715
JP 07067143	A2	19950314	JP 1994-164143	19940715
BR 9402834	A	19950613	BR 1994-2834	19940715
US 6180375	B1	20010130	US 1994-275526	19940715

PRIORITY APPLN. INFO.: GB 1993-14780 A 19930715

AB A purified xylanase derived from B. pumilus PRL B12 is disclosed. This xylanase is efficient for use in the biobleaching of wood pulp, permitting a strong redn. in the quantity of chlorine used and AOX compds. produced

in classical and ECF wood pulp bleaching sequences as well as the quantity of ozone used in TCF sequences. The gene coding for the xylanase was isolated and purified and used to construct an expression vector therefor.
A recombinant host strain of B. licheniformis is also disclosed which is efficient for expressing heterologous enzymes, including the xylanase when transformed by the expression vector. The pH and temp. optima and pI of the xylanase were detd. An alk. protease **deletion** **mutant** of B. licheniformis was prepd. and used for expression of the xylanase gene as well as for a no. of other enzyme genes.

L5 ANSWER 11 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 94:450031 SCISEARCH
THE GENUINE ARTICLE: NW965
TITLE: GENE TARGETING IN A KLEBSIELLA SP BY FUSARIC ACID SELECTION AND THE USE OF TEMPERATURE-SENSITIVE REPLICON
AUTHOR: SUGINO H; AZAKAMI H; ARAI S; MUROOKA Y (Reprint)
CORPORATE SOURCE: KURUME UNIV, SCH MED, DEPT BACTERIOL, 67 ASAHI MACHI, KURUME, FUKUOKA 830, JAPAN (Reprint); KURUME UNIV, SCH MED, DEPT BACTERIOL, KURUME, FUKUOKA 830, JAPAN; HIROSHIMA UNIV, FAC ENGN, DEPT FERMENTAT TECHNOL, HIGASHIHIROSHIMA 724, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF FERMENTATION AND BIOENGINEERING, (1994) Vol. 77, No. 6, pp. 712-715.
ISSN: 0922-338X.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 16
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Two previously described methods for gene targeting (replacement) in Escherichia coil were applied to the disruption of the maoCA operon in Klebsiella aerogenes. These techniques involve plasmid-chromosomal integration, resolution of the integrated intermediate and segregation (i) screened using fusaric acid for the counter selection of plasmid replicons carrying the gene for tetracycline resistance, or (ii) by using a temperature sensitive replicon. Both methods were found to be effective to create the desired chromosomal **mutants** in a Klebsiella strain after some **modifications** of the original experimental protocols, and may serve as tools for gene targeting studies in Klebsiella and related species.

L5 ANSWER 12 OF 16 MEDLINE
ACCESSION NUMBER: 93346376 MEDLINE
DOCUMENT NUMBER: 93346376 PubMed ID: 8344920
TITLE: Sequencing of the amylopullulanase (apu) gene of Thermoanaerobacter ethanolicus 39E, and identification of the active site by site-directed mutagenesis.
AUTHOR: Mathupala S P; Lowe S E; Podkovyrov S M; Zeikus J G
CORPORATE SOURCE: Department of Biochemistry, Michigan State University, East Lansing 48824.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Aug 5) 268 (22) 16332-44.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-M97665
ENTRY MONTH: 199[redacted]9
ENTRY DATE: Entered STN: 19930924
Last Updated on STN: 20000525
Entered Medline: 19930907

AB The complete nucleotide sequence of the gene encoding the dual active amylopullulanase of Thermoanaerobacter ethanolicus 39E (formerly Clostridium thermohydrosulfuricum) was determined. The structural gene (apu) contained a single open reading frame 4443 base pairs in length, corresponding to 1481 amino acids, with an estimated molecular weight of 162,780. Analysis of the deduced sequence of apu with sequences of alpha-amylases and alpha-1,6 debranching enzymes enabled the identification of four conserved regions putatively involved in substrate binding and in catalysis. The conserved regions were localized within a 2.9-kilobase pair gene fragment, which encoded a M(r) 100,000 protein that maintained the dual activities and thermostability of the native enzyme. The catalytic residues of amylopullulanase were tentatively identified by using hydrophobic cluster analysis for comparison of amino acid sequences of amylopullulanase and other amylolytic enzymes. Asp597, Glu626, and Asp703 were individually **modified** to their respective amide form, or the alternate acid form, and in all cases both alpha-amylase and **pullulanase** activities were lost, suggesting the possible involvement of 3 residues in a catalytic triad, and the presence of a putative single catalytic site within the enzyme. These findings substantiate amylopullulanase as a new type of amylosaccharidase.

L5 ANSWER [redacted] OF 16 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 95020627 MEDLINE
DOCUMENT NUMBER: 95020627 PubMed ID: 7934912
TITLE: The role of the lipoprotein sorting signal (aspartate +2) in **pullulanase** secretion.
AUTHOR: Poquet I; Kornacker M G; Pugsley A P
CORPORATE SOURCE: Unite de Genetique Moleculaire (CNRS-URA1149), Institut Pasteur, Paris, France.
SOURCE: MOLECULAR MICROBIOLOGY, (1993 Sep) 9 (5) 1061-9.
Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19941222
Entered Medline: 19941114

QR74.M65

AB The analyses of hybrid proteins and of **deletion** and insertion mutations reveal that the only amino acid at the amino-proximal end of the cell surface lipoprotein **pullulanase** that is specifically required for its extracellular secretion is an aspartate at position +2, immediately after the fatty acylated amino-terminal cysteine. To see whether the requirement for this amino acid is related to its proposed role as a cytoplasmic membrane lipoprotein sorting signal, we used sucrose gradient flotation analysis to determine the subcellular location of pullulanase **variants** (with or without the aspartate residue) that accumulated in cells lacking the **pullulanase** -specific secretion genes. A non-secretable **pullulanase** variant with a serine at position +2 cofractionated mainly with the major peak of outer membrane porin. In contrast, most (55%) of a pullulanase **variant** with an aspartate at position +2 cofractionated with slightly lighter fractions that contained small proportions of both outer membrane porin and the cytoplasmic membrane marker NADH oxidase. Only 5% of this **pullulanase variant** cofractionated with the major NADH oxidase peak, while the rest (c. 40%) remained at the bottom of the gradient in fractions totally devoid of

porin ... NADH oxidase. When analysed by sedimentation through sucrose grad... , howeve... large proportion of this **vari...** was recov... from fractions near the top of the gradient that also contained the m... NADH oxidase peak. When this peak fraction was applied to a float... gradient the **pullulanase** activity remained at the bott... le the NADH oxidase floated to the top.(ABSTRACT TRUNCATED AT 250 W

L5 ANSWE... OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSIO... ER: 93:162347 SCISEARCH
THE GENUI... ICLE: KQ909
TITLE: MUTAGENESIS OF CELLULASE EGZ FOR STUDYING THE GENERAL PROTEIN SECRETORY PATHWAY IN ERWINIA-CHRYSANTHEMI
AUTHOR: PY B; CHIPPAUX M; BARRAS F (Reprint)
CORPORATE ... E: CNRS, LCB, 31 CHEM JOSEPH AIGUIER, F-13277 MARSEILLE 9, FRANCE
COUNTRY OF ... HOR: FRANCE
SOURCE: MOLECULAR MICROBIOLOGY, (MAR 1993) Vol. 7, No. 5, pp. 785-793.
ISSN: 0950-382X.
DOCUMENT ... : , Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 48
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB E... ellular secretion of endoglucanase Z (EGZ) from Erwinia ch... emi is mediated by the so-called Out general secretion pathway a... umably, involves recognition of EGZ-carried structural info... on by one or more of the Out proteins. Investigating the r... nips between structure and secretability of EGZ was the purpose o... esent work. EGZ is made of two independent domains, located at t... C-proximal sides, separated by a Ser/Thr-rich region, which are r... le for catalysis and cellulose-binding, respectively. The ex... st... of a secretion region ('targeting signal') was investigated by s... the secretability of **modified** EGZ derivatives. These r... from **deletion** or peptide insertion and were designed by u... domain organization cited above as a guide. Catalytic and/or c... -binding tests showed that all proteins exhibited at least a f... EGZ domain while immunoblot analyses confirmed that neither the i... s nor the **deletions** led to grossly misfolded proteins. I... ct, all of the proteins lost their secretability in E. c... mi. This suggested that at least two secretion motifs existed, o... within each functional domain. The role of the Ser/Thr-rich l... ion was subsequently tested. Accordingly, two proteins c... a linker region whose length was increased by the addition of 8 a... ditional residues and one protein lacking the linker region were s... ll three exhibited endoglucanase activity and cellulose-binding a... confirming the independence of the domains within the context of E... ccharide interaction. In contrast, none was secreted by E. c... mi. Collectively, our results with EGZ (i) suggest the c... of multiple secretion-related sites either acting sequentially o... a single three-dimensional secretion signal, (ii) show that s... ity is not determined by either one of the two functional d... one, and (iii) reveal that the linker region plays a role in s... We propose that all EGZ derivatives were impaired in the l... on step, the nature of which is discussed.

L5 A... OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESS... R: 92:241937 SCISEARCH
THE G... CLE: HM899
TITLE: CLONING AND CHARACTERIZATION OF A GENE REQUIRED FOR THE SECRETION OF EXTRACELLULAR ENZYMES ACROSS THE OUTER-MEMBRANE BY XANTHOMONAS-CAMPESTRIS PV CAMPESTRIS
AUTHOR: HU N T (Reprint); HUNG M N; CHIOU S J; TANG F; CHIANG D C;

HUANG H Y; WU C Y
CORPORATE SOURCE: NATL CHUNG HSING UNIV, AGR BIOTECH LABS, 250 KUO KUANG RD, TAICHUNG 40227, TAIWAN (Reprint); NATL CHUNG HSING UNIV, GRAD INST BIOL, TAICHUNG 40227, TAIWAN
COUNTRY OF AUTHOR: TAIWAN
SOURCE: JOURNAL OF BACTERIOLOGY, (APR 1992) Vol. 174, No. 8, pp. 2679-2687.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 51
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB ...hogenic **mutants** of Xanthomonas campestris pv. ca... ...s, generated from transposon mutagenesis, accumulated ex... ...lular polygalacturonate lyase, alpha-amylase, and endoglucanase in th... ...iplasm. The transposon Tn5 was introduced by a mobilizable, su... ...l plasmid, pSUP2021 or pEYDG1. Genomic banks of wild-type X. c... ...ris pv. campestris, constructed on the broad-host-range, m... ...ble cosmid pLAFR1 or pLAFR3, were conjugated with one of the m... ...designated XC1708. Recombinant plasmids isolated by t... ...lity to complement XC1708 can be classified into two categories. O... ...esented by pLASC3, can complement some **mutants**, whereas th... ...r, represented by a single plasmid, pLAHH2, can complement all of th... ...er **mutants**. Restriction mapping showed that the two r... ...nant plasmids shared an EcoRI fragment of 8.9 kb. Results from s... ...ng, **deletion** mapping, and mini-Mu insertional mutation c... ...9-kb EcoRI fragment suggested that a 4.2-kb fragment was s... ...nt to complement the **mutant** XC1708. Sequence analysis o... ...2-kb fragment revealed three consecutive open reading frames (... ...F1, ORF2, and ORF3. Hybridization experiments showed that Tn5 i... ...nome of XC1708 and other **mutants** complemented by pLASC3 w... ...ted in ORF3, which could code for a protein of 83.5 kDa. A signal p... ...e II processing site was identified at the N terminus of the p... ...d amino acid sequence. Sequence homology of 51% was observed b... ...the amino acid sequences predicted from ORF3 and the pulD gene of K... ...la species.

L5 ... 6 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 92:334813 SCISEARCH
THE GEN ARTICLE: HV865
TITLE: THE AEROMONAS-HYDROPHILA EXEE GENE, REQUIRED BOTH FOR PROTEIN SECRETION AND NORMAL OUTER-MEMBRANE BIOGENESIS, IS A MEMBER OF A GENERAL SECRETION PATHWAY
AUTHOR: JIANG B; HOWARD S P (Reprint)
CORPORATE SOURCE: MEM UNIV NEWFOUNDLAND, ST JOHNS A1B 3V6, NEWFOUNDLAND, CANADA
COUNTRY OF AUTHOR: CANADA
SOURCE: MOLECULAR MICROBIOLOGY, (MAY 1992) Vol. 6, No. 10, pp. 1351-1361.
ISSN: 0950-382X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 47
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB ...romonas hydrophila Tn5-751 insertion **mutant** L1.97 is un... ...secrete extracellular proteins, and is fragile because of de... ...assembly of its outer membrane. A KpnI 4.1 kb fragment, which com... ...nts this **mutant** when supplied with an exogenous prom..., was isolated and sequenced. It contains two complete genes, exeE and ..., plus fragments of two others and may form part of an operon. The

exeE ... exeF open reading frames encode 501-residue M(r) 55882 and
388-... due M(r) ...31 proteins, respectively. The ... genes were expressed
in ... and their initiation codons verified by deletion
ana... . Tn5-751 had inserted near the centre of the exeE gene in the
L1... rain. Subclones of the KpnI 4.1 kb fragment which contained only
the ... gene fully complemented the mutation, indicating that its
func... is required both for extracellular secretion and outer membrane
asse... . ExeE and ExeF are highly similar to other proteins which have
been ...wn to be involved in extracellular secretion, suggesting that an
addi... al export apparatus beyond that required for inner membrane
tra... ...etion may be part of the physiology of many Gram-negative
bac... ..

=> d l5 ibib ab 1-2

L5 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
ACCESSION NUMBER: 1995:702381 CAPLUS
DOCUMENT NUMBER: 123:142344
TITLE: Safety evaluation of **pullulanase** enzyme preparation derived from Bacillus licheniformis containing the **pullulanase** gene from Bacillus deramificans
AUTHOR(S): Modderman, John P.; Foley, Holly H.
CORPORATE SOURCE: Keller and Heckman, Washington, DC, 20001, USA
SOURCE: Regul. Toxicol. Pharmacol. (1995), 21(3), 375-81
CODEN: RTOPDW; ISSN: 0273-2300
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Pullulanase** enzyme is an amylopectin debranching enzyme used in starch hydrolysis. This article describes studies conducted to investigate the safety of a **pullulanase** enzyme prepn. produced by a strain of Bacillus licheniformis that has been transformed by introduction of genetic material from another Bacillus species, **B. deramificans**. A 4-wk dietary toxicity study in rats was conducted in which test animals received **pullulanase** in the feed at concns. of 0.2, 1.0, and 5.0%. No adverse treatment-related effects were obsd. Lack of genetic toxicity potential was demonstrated by the results of a bacterial mutation assay in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538, in an in vitro histidine forward mutation study in mouse lymphoma cells, and in in vivo mouse bone marrow chromosome aberration and micronucleus assays. The enzyme prepn. also has been shown to be a nonirritant in eye and primary dermal irritation tests in rabbits and is nontoxic by inhalation exposure. Finally, the genetically altered B. licheniformis has been demonstrated to be nonpathogenic upon single i.p. injection to rats of both live and killed cells at doses up to 1011 cells/kg. The results of these studies demonstrate that the enzyme prepn. may be considered safe when employed in starch processing.

L5 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:624994 CAPLUS
DOCUMENT NUMBER: 121:224994
TITLE: A novel **pullulanase** that is thermostable under acid conditions and cloning and expression of the gene encoding it
INVENTOR(S): DeWeer, Philippe; Amory, Antoine
PATENT ASSIGNEE(S): Solvay et Cie., Belg.
SOURCE: Eur. Pat. Appl., 61 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 605040	A1	19940706	EP 1993-203593	19931220
EP 605040	B1	19990811		

R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, PT

BE 1006483	A3	19940913	BE 1992-1156		19921228
BE 1007313		19950516	BE 1993-744		19930715
BE 1007723	A6	19951010	BE 1993-1278		19931119
AT 183236	E	19990815	AT 1993-203593		19931220
ES 2137222	T3	19991216	ES 1993-203593		19931220
FI 9305900	A	19940629	FI 1993-5900		19931228
CN 1090325	A	19940803	CN 1993-121736		19931228
CN 1061089	B	20010124			
JP 06217770	A2	19940809	JP 1993-337202		19931228
CA 2112028	AA	19940629	CA 1993-2112028		19931229
AU 9352759	A1	19940707	AU 1993-52759		19931230
AU 686574	B2	19980212			
US 5721127	A	19980224	US 1995-474140		19950607
US 5721128	A	19980224	US 1995-477630		19950607
US 5731174	A	19980324	US 1995-472293		19950607
US 5736375	A	19980407	US 1995-474545		19950607
US 6074854	A	20000613	US 1997-996733		19971223
AU 9864831	A1	19980730	AU 1998-64831		19980511

PRIORITY APPLN. INFO.:

BE 1992-1156	A	19921228
BE 1993-744	A	19930715
BE 1993-1278	A	19931119
US 1993-174893	B1	19931228
US 1995-472293	A1	19950607

AB A novel **pullulanase** that is heat-stable at acid pHs is obtained from Bacillus and the gene encoding it is cloned and expressed for manuf. of the enzyme for processing polysaccharides. The enzyme has a temp. optimum of 55-65.degree. at pH 4.3 and retains >80% of its activity in the pH range 3.8-4.9. An isolate of Bacillus deramificans capable of hydrolyzing a pullulan deriv. at 37.degree.; the strain (**B. deramificans** T89.117D) was not itself heat-tolerant. The enzyme accumulated in the medium and was purified 10-fold (32% yield) from cultures grown on yeast ext./potato starch medium by centrifugation, heat treatment, acetone pptn., and ion-exchange chromatog. The gene was cloned by expression from a Sau3A partial bank in pBR322. The cloned gene was expressed in a Bacillus licheniformis host from which the alk. proteinase gene had been deleted using either an autonomously replicating or

L8 ANSWER 14 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1988:469474 CAPLUS
DOCUMENT NUMBER: 109:69474
TITLE: Functional analysis of the starch debranching enzyme **pullulanase**
AUTHOR(S): McPherson, Michael J.; Charalambous, Bambos M.
CORPORATE SOURCE: Biotechnol. Unit, Univ. Leeds, Leeds, LS2 9JT, UK
SOURCE: Biochem. Soc. Trans. (1988), 16(5), 723-4
CODEN: BCSTB5; ISSN: 0300-5127
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The predicted amino acid sequences of **pullulanases** from **Klebsiella pneumoniae** strains W70 and FG9 are very similar and provide a basis for the design of expts. to exam. **pullulanase** function. Proteolytic digestion studies and computer-based sequence anal. are being used to define a functional core **pullulanase**. Computer searches identified homol., as expected, between the N-terminal region of **pullulanase** and a range of collagens and collagen-like proteins. No extensive homologies were detected with any protein sequences in the database, although a no. of significant localized identities with .alpha.-amylases were revealed. A series of 5 amylase matrixes, corresponding to 5 regions of sequence conservation within microbial .alpha.-amylases, were constructed from aligned sequence data to search specific protein sequences with these 5 amylase matrixes; the expected pattern of conserved regions was clearly identified within all the .alpha.-amylase and cyclodextrin glucantransferase sequences tested. The same pattern of conserved sequences was found within the C-terminal half of **pullulanase**. Structural evidence, from Taka-amylase suggested certain conserved regions include residues (such as glutamate-230 and aspartate-297) implicated in substrate binding and catalysis. **Pullulanase** and .alpha.-amylases may thus have functional and mechanistic similarity. An active **variant** resulting from .gamma.-chymotrypsin treatment was also characterized. Protein sequencing data showed that 170 residues were removed from the N-terminus and preliminary data using starch as substrate suggested that the debranching activity of this **variant** is .apprx.30% higher than that of the native enzyme.

L8 ANSWER 12 OF 18 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 7
ACCESSION NUMBER: 1989:611737 CAPLUS
DOCUMENT NUMBER: 111:211737
TITLE: Biosynthesis and secretion of **pullulanase**, a lipoprotein from **Klebsiella aerogenes**
AUTHOR(S): Murooka, Yoshikatsu; Ikeda, Ryuji
CORPORATE SOURCE: Fac. Eng., Hiroshima Univ., Higashi-Hiroshima, 724, Japan
SOURCE: J. Biol. Chem. (1989), 264(29), 17524-31
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A **mutant pullulanase** gene was constructed, by site-directed mutagenesis, in which the cysteine residue in a pentapeptide sequence, Leu16-Leu-Ser-Gly-Cys20, within the NH2-terminal region of **pullulanase** from K. aerogenes is replaced by serine (Ser20). The **modification**, processing, and subcellular localization of the **mutant pullulanase** were studied. Labeling studies with [3H]palmitate and immunopptn. with mouse antiserum raised against **pullulanase** showed that the wild form of both the extracellular and intracellular **pullulanases** contained lipids, whereas the **mutant** enzyme was not **modified** with lipids. Only the Cys20 was **modified** with glyceryl lipids. The bulk of **mutant pullulanase** was located in the periplasm, but a portion of the unmodified, **mutant pullulanase** was secreted into the medium. **Mutant pullulanases** from the extracellular and the periplasm were purified and their NH2-terminal sequences were detd. Both the **mutant pullulanases** were cleaved between residues of Ser13 and Leu14 which is 6 amino acid residues upstream of the lipid **modified pullulanase** cleavage site. This new cleavage was resistant to globomycin, an inhibitor of the prolipoprotein signal peptidase of Escherichia coli. The pentapeptide sequence apparently plays an important role in maturation and translocation of **pullulanase** in K. aerogenes. However, the **modification** of **pullulanase** with lipids seems to be not essential for export of the enzyme across the outer membrane.

L8

L8 ANSWER 6 OF 18 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
ACCESSION NUMBER: 1995:311056 CAPLUS
DOCUMENT NUMBER: 122:100484
TITLE: Random mutagenesis of **pullulanase** from **Klebsiella aerogenes** for studies of the structure and function of the enzyme
AUTHOR(S): Yamashita, Mitsuo; Kinoshita, Takuya; Ihara, Michiko; Mikawa, Tomomi; Murooka, Yoshikatu
CORPORATE SOURCE: Faculty Engineering, Hiroshima University, Hiroshima, 724, Japan
SOURCE: J. Biochem. (Tokyo) (1994), 116(6), 1233-40
CODEN: JOBIAO; ISSN: 0021-924X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To study the structure and function of **pullulanase** from K. aerogenes, a method involving random mutagenesis of the entire gene for **pullulanase** was used. Out of 50,000 clones screened at high temp., 7 genes for **mutant** proteins were identified by DNA sequencing. The amino acid substitutions in the 7 **mutant** proteins were clustered on the N-terminal side of the 4 conserved regions found in .alpha.-amylases. These **mutant pullulanases** were classified into 2 types: those whose catalytic activity was altered and those whose thermal stability was increased. The results presented here and in previous reports suggest that **pullulanase** from K. aerogenes has similar active sites to those of .alpha.-amylases with the 4 conserved regions, as well as another substrate-binding site closer to the N-terminus. The plate method used for isolation of thermostable **variants** may be applicable to the generation of useful **variants** of other enzymes.

L8 ANSWER 3 OF 18 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
ACCESSION NUMBER: 1997:738051 CAPLUS
DOCUMENT NUMBER: 128:72332
TITLE: Amino acid residues specific for the catalytic action towards .alpha.-1,6-glucosidic linkages in Klebsiella **pullulanase**
AUTHOR(S): Yamashita, Mitsuo; Matsumoto, Dai; Murooka, Yoshikatsu
CORPORATE SOURCE: Department of Biotechnology, Graduate School of Engineering, Osaka University, Osaka, 565, Japan
SOURCE: J. Ferment. Bioeng. (1997), 84(4), 283-290
CODEN: JFBIEX; ISSN: 0922-338X
PUBLISHER: Society for Fermentation and Bioengineering, Japan
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Mutations were introduced at residues His607, Asp677, His682, and His833 in **pullulanase** from **Klebsiella aerogenes** in order to probe the role of these amino acid residues, which are located in the four conserved regions of the .alpha.-amylase family, in the action of the enzyme towards .alpha.-1,6-glucosidic linkages. For the mutations, His was replaced by Asn and Ala, and Asp by Asn and Ser. Amino acid substitutions for His607, Asp677, or His833 resulted in complete loss of enzyme activity. In contrast, the mutations at His682 still retained their activities. The binding affinity of these **variants** for .alpha.- or .beta.-cyclodextrin (CD), which are competitive inhibitors for **pullulanase**, was measured using an .alpha.-CD Sepharose column. The mutations at His833 did not change the binding affinity for .alpha.-CD, whereas the mutations at His607 or Asp677 resulted in these two **variants** losing their binding ability towards pullulan. These results suggest that in Klebsiella **pullulanase**, His607 and Asp677 participate in substrate binding and His833 is involved in catalysis, but His682 may not be in the active site. We also found new amino acid consensus sequences specific for starch debranching enzymes in two oligo-1,6-glucosidases, several **pullulanases**, and an isoamylase. Two amino acid residues in the predicted consensus region of Klebsiella **pullulanase**, Tyr559 and Tyr564, were replaced by Ala or Phe. The Tyr559 **variants** resulted in complete loss of **pullulanase** activity without seriously affecting the binding affinities for .alpha.-CD and pullulan. The mutations at Tyr564 did not completely inactivate the enzymes but dramatically decreased the activity. Thus, the region in Klebsiella **pullulanase** that includes Tyr559-Tyr564 probably participates in catalysis specific towards .alpha.-1,6-glucosidic linkages in starch debranching enzymes.

=> d l4 ibib ab 1-14

L4 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:526181 CAPLUS
DOCUMENT NUMBER: 135:118784
TITLE: **Bacillus** deramificans **pullulanase** variants and methods for preparing such variants with predetermined properties
INVENTOR(S): Svendsen, Allan; Andersen, Carsten; Vedel Borchert, Torben
PATENT ASSIGNEE(S): Novozymes A/S, Den.
SOURCE: PCT Int. Appl., 195 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001051620	A2	20010719	WO 2001-DK20	20010112

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: DK 2000-45 A 20000112
US 2000-514599 A 20000228

AB The present invention relates to a method for producing a variant of a parent **pullulanase**, the variant having at least one altered property as compared to the parent **pullulanase**. The altered properties include stability (e.g., thermostability), pH dependent activity, substrate cleavage pattern, specific activity of cleavage, substrate specificity, such as higher activity of isoamylase activity and /or substrate binding. Thirty-one substitution or **deletion** mutants of **Bacillus** deramificans **pullulanase** were made by PCR and tested after transformation and fermn. in **Bacillus** subtilis. The invention also relates to **pullulanase** variants and to the use of **pullulanase** variants of the invention for use in particular starch conversion processes.

L4 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:68546 CAPLUS
DOCUMENT NUMBER: 132:104698
TITLE: Glucoamylase variants with improved specific activity and/or thermostability
INVENTOR(S): Nielsen, Bjarne Ronfeldt; Svendsen, Allan; Pedersen, Henrik; Vind, Jesper; Hendriksen, Hanne Vang; Frandsen, Torben Peter
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 117 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000004136	A1	20000127	WO 1999-DK392	19990709

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9947699	A1	20000207	AU 1999-47699	19990709
EP 1097196	A1	20010509	EP 1999-931029	19990709

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

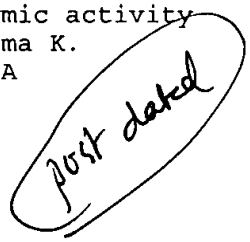
PRIORITY APPLN. INFO.:

DK 1998-937	A	19980715
DK 1998-1667	A	19981217
WO 1998-DK937	W	19980715
WO 1998-DK1667	W	19981217
WO 1999-DK392	W	19990709

AB The invention relates to a variant of a parent fungal glucoamylase, which exhibits improved thermal stability and/or increased specific activity using saccharide substrates. The x-ray structure and/or model-build structure of Aspergillus awamori variant X100 glucoamylase was subjected to mol. dynamics simulations to identify regions important for temp.-stable activity. The **truncated** G1 glucoamylase from Aspergillus niger was modified by (1) random mutagenesis, (2) localized random, doped mutagenesis, or (3) PCR shuffling spiked with DNA oligonucleotides in order to prep. variants having improved thermostability compared to the parent enzyme. Such glucoamylase variants have use in starch saccharification, oligosaccharide prodn., specialty syrups, producing ethanol for fuel, producing beverages, and producing org. compds. (citric acid, ascorbic acid, lysine, glutamic acid).

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:577030 CAPLUS
DOCUMENT NUMBER: 131:196365
TITLE: N-terminal-**truncated** analogs of bacterial **pullulanases** retaining normal enzymic activity
INVENTOR(S): Miller, Brian S.; Shetty, Jayarama K.
PATENT ASSIGNEE(S): Genencor International, Inc., USA
SOURCE: PCT Int. Appl., 49 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:



PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9945124	A2	19990910	WO 1999-US4627	19990303
WO 9945124	A3	19991118		

W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9929801	A1	19990920	AU 1999-29801	19990303
BR 9908422	A	20001031	BR 1999-8422	19990303
EP 1060253	A2	20001220	EP 1999-911068	19990303

R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE, FI

PRIORITY APPLN. INFO.: US 1998-34630 A 19980304
WO 1999-US4627 W 19990303

AB **Pullulanases** from **Bacillus** and Klebsiella that retain normal 1,6-.alpha.-glycosidase activity despite having **truncations** of up to 300 amino acids from the N-terminal domain, optionally with further amino acid substitutions, and that may be useful in the starch industry are described. The present invention provides methods for producing the modified **pullulanase**, enzymic compns. comprising the modified **pullulanase**, and methods for the saccharification of starch comprising the use of the enzymic compns. Expression of the **Bacillus** deramificans **pullulanase** gene in B. licheniformis hosts lacking the Carlsberg subtilisin and endopeptidase Glu-C resulted in the appearance of a series of N-terminal **deletions** of the **pullulanase**. Saccharification of starch with mixts. of glucoamylase (20%) and the **pullulanases** (80%) led to the saccharification of the starch without the formation of disaccharides.

L4 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:794818 CAPLUS
DOCUMENT NUMBER: 130:106926
TITLE: **Pullulanase** mutants of **Bacillus** strain KSM-AP1378 for preparation of detergents and starch-saccharifying agents
INVENTOR(S): Sumitomo, Nobuyuki; Hatada, Yuji; Ichimura, Takashi; Saito, Kazuhiro; Kawai, Shuji; Ito, Susumu
PATENT ASSIGNEE(S): Kao Corp., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 19 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 10327868	A2	19981215	JP 1997-141596	19970530

AB Prepn. of mutants of **pullulanase** of **Bacillus** strain KSM-AP1378 by **deletion** or substitution mutation at 443-Met and/or 557-Ala to improved their resistance to oxidizing agents; and use of the mutants for the prepn. of detergents and starch-saccharifying agents are described. The **pullulanase** is derived from the domain 1023-Met.apprx.1820-Asp of the 1938-amino-acid amylopullulanase of **Bacillus** strain KSM-AP1378. Prepn. of single mutants M443A, M443E, M443I, M443L, M443N, M443R, M443S, and M443V; prepn. of double mutants such as M443L/A557C; their stability in the presence of H2O2; and the washing ability of a detergent compn. contg. them were also shown. Also claimed are the detergent and saccharifying agents contg. the **pullulanase** mutants and other enzymes such as .alpha.-amylase, glucoamylase, etc.

L4 ANSWER 5 OF 14 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 1998:183379 SCISEARCH
THE GENUINE ARTICLE: YZ056
TITLE: Conversion of neopullulanase-alpha-amylase from Thermoactinomyces vulgaris R-47 into an amylopullulanase-type enzyme
AUTHOR: Ibuka A; Tonozuka T; Matsuzawa H; Sakai H (Reprint)
CORPORATE SOURCE: UNIV SHIZUOKA, SCH FOOD & NUTR SCI, 52-1 YADA, SHIZUOKA

422, JAPAN (Reprint); UNIV SHIZUOKA, SCH FOOD & NUTR SCI, SHIZUOKA 422, JAPAN; UNIV TOKYO, DEPT BIOTECHNOL, BUNKYO KU, TOKYO 113, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF BIOCHEMISTRY, (FEB 1998) Vol. 123, No. 2, pp. 275-282.
Publisher: JAPANESE BIOCHEMICAL SOC, ISHIKAWA BLDG-3F, 25-16 HONGO-5-CHOME, BUNKYO-KU, TOKYO 113, JAPAN.
ISSN: 0021-924X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 31

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB TVA I, an alpha-amylase from Thermoactinomyces vulgaris R-47, is a versatile enzyme which hydrolyzes the alpha-(1-->4)-glucosidic linkages of pullulan to produce panose, known as neopullulanase activity, and the alpha-(1-->6)-glucosidic linkages of certain oligosaccharides, We modified the Ala-357, Gin-359, and Tyr-360 residues located in region II, one of the four regions conserved in a-amylase family enzymes, and **deleted** 11 consecutive amino acid residues located after the C-terminus of region II of the TVA I sequence by means of site-directed mutagenesis, The action pattern of the mutated enzyme for pullulan was greatly altered and it hydrolyzed mainly the alpha-(1-->6)-glucosidic linkages of pullulan to produce maltotriose, while the action patterns for starch and maltooligosaccharides were almost identical to those of the wild-type enzyme, This means that the mutated TVA I has lost the neopullulanase activity, and thus can be designated as an amylopullulanase-type enzyme, The k(cat)/K-m value of the mutated enzyme for alpha-(1-->6)-glucosidic linkages was virtually unaltered, while that for alpha-(1-->4)-glucosidic linkages was about 100 times smaller than that of the wild-type enzyme.

L4 ANSWER 6 OF 14 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 97:844280 SCISEARCH
THE GENUINE ARTICLE: YF004
TITLE: Cloning and sequence of a type I **pullulanase** from an extremely thermophilic anaerobic bacterium, Caldicellulosiruptor saccharolyticus
AUTHOR: Albertson G D; McHale R H; Gibbs M D; Bergquist P L (Reprint)
CORPORATE SOURCE: MACQUARIE UNIV, RES OFF, SYDNEY, NSW 2109, AUSTRALIA (Reprint); UNIV AUCKLAND, CTR GENE TECHNOL, AUCKLAND 1, NEW ZEALAND; UNIV AUCKLAND, SCH MED, DEPT MOL MED, AUCKLAND, NEW ZEALAND; MACQUARIE UNIV, SCH BIOL SCI, SYDNEY, NSW 2109, AUSTRALIA
COUNTRY OF AUTHOR: AUSTRALIA; NEW ZEALAND
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-GENE STRUCTURE AND EXPRESSION, (9 OCT 1997) Vol. 1354, No. 1, pp. 35-39.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0167-4781.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 18

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A gene coding for a **pullulanase** from the obligately anaerobic, extremely thermophilic bacterium Caldicellulosiruptor saccharolyticus has been cloned in Escherichia coli. It consists of an open reading frame (pulA) of 2478 bp which codes for an enzyme of 95732 Da and is flanked by two other open reading frames. A **truncated**

activity staining gel, while recombinant E. coli expressed a single amylase with a mo[redacted]wt. of 220 kDa (AapT), which c[redacted]esponded to the mol. wt. calcd. from the open reading frame of aapT. The optimum temp. for the activities of AapT-1 (85 kDa) and AapT-2 (135 kDa) was 70.degree.C, which is the same as that of the full-sized AapT (220 kDa) from E. coli. In contrast, the optimum pH for the activities of AapT-1 and AapT-2 were pH 7.0 and pH 8.0, resp., whereas that of the AapT (220 kDa) was pH 9.0. These observations indicated that the optimum pH for the activity of AapT was changed from the alk. to the neutral region when the enzyme was expressed in a lower mol. wt. **truncated** form. Furthermore, amino acid sequence alignment suggested that AapT was **truncated** in its C terminal region. Therefore, the noncatalytic C-terminal region may be responsible for the high optimum pH of the enzyme activity. In addn., activity staining and further anal. of AapT from the original strain, **Bacillus** sp. XAL601, showed glycosylation of the enzyme.

L4 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:372926 CAPLUS
DOCUMENT NUMBER: 122:153390
TITLE: **Bacillus** xylanase and gene, expression vectors for the xylanase and other proteins, expression hosts therefor, and use of xylanase in pulp bleaching
INVENTOR(S): De, Buyl Eric; Lahaya, Andree; Ledoux, Pierre; Amory, Antoine; Detroz, Rene; Andre, Christophe; Vetter, Roman
PATENT ASSIGNEE(S): Solvay et Cie., Belg.
SOURCE: Eur. Pat. Appl., 78 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 634490	A1	19950118	EP 1994-202002	19940711
EP 634490	B1	20010912		
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, PT, SE				
GB 2279955	A1	19950118	GB 1993-14780	19930715
GB 2279955	B2	19980218		
AU 9467432	A1	19950127	AU 1994-67432	19940713
AU 687808	B2	19980305		
CA 2128050	AA	19950116	CA 1994-2128050	19940714
NO 9402652	A	19950116	NO 1994-2652	19940714
FI 9403389	A	19950116	FI 1994-3389	19940715
JP 07067637	A2	19950314	JP 1994-164143	19940715
BR 9402834	A	19950613	BR 1994-2834	19940715
US 6180382	B1	20010130	US 1994-275526	19940715

PRIORITY APPLN. INFO.: GB 1993-14780 A 19930715

AB A purified xylanase derived from B. pumilus PRL B12 is disclosed. This xylanase is efficient for use in the biobleaching of wood pulp, permitting a strong redn. in the quantity of chlorine used and AOX compds. produced in classical and ECF wood pulp bleaching sequences as well as the quantity of ozone used in TCF sequences. The gene coding for the xylanase was isolated and purified and used to construct an expression vector therefor. A recombinant host strain of B. licheniformis is also disclosed which is efficient for expressing heterologous enzymes, including the xylanase when transformed by the expression vector. The pH and temp. optima and pI of the xylanase were detd. An alk. protease **deletion** mutant of B.

licheniformis was prepd. and used for expression of the xylanase gene as well as for a no. other enzyme genes.

L4 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
ACCESSION NUMBER: 1995:804017 CAPLUS
DOCUMENT NUMBER: 123:310050
TITLE: Extracellular secretion of **pullulanase** is unaffected by minor sequence changes but is usually prevented by adding reporter proteins to its N- or C-terminal end
AUTHOR(S): Sauvonnet, Nathalie; Poquet, Isabelle; Pugsley, Anthony P.
CORPORATE SOURCE: Unite Genetique Moleculaire, Institut Pasteur, Paris, 75724, Fr.
SOURCE: J. Bacteriol. (1995), 177(18), 5238-46
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Linker insertions in the **pullulanase** structural gene (pulA) were examd. for their effects on **pullulanase** activity and cell surface localization in Escherichia coli carrying the cognate secretion genes from Klebsiella oxytoca. Of the 23 insertions, 11 abolished **pullulanase** activity but none were found to prevent secretion. To see whether more drastic changes affected secretion, we fused up to five reporter proteins (E. coli periplasmic alk. phosphatase, E. coli periplasmic maltose-binding protein, periplasmic TEM .beta.-lactamase, Erwinia chrysanthemi extracellular endoglucanase Z, and **Bacillus** subtilis extracellular levansucrase) to three different positions in the **pullulanase** polypeptide: close to the N terminus of the mature protein, at the C terminus of the protein, or at the C terminus of a **truncated pullulanase** variant lacking the last 256 amino acids. Only 3 of the 13 different hybrids were efficiently secreted: 2 in which .beta.-lactamase was fused to the C terminus of full-length or **truncated pullulanase** and 1 in which maltose-binding protein was fused close to the N terminus of **pullulanase**. Affinity-purified endoglucanase-**pullulanase** and **pullulanase**-endoglucanase hybrids exhibited apparently normal levels of **pullulanase** activity, indicating that the conformation of the **pullulanase** segment of the hybrid had not been dramatically altered by the presence of the reporter. However, **pullulanase**-endoglucanase hybrids were secreted efficiently if the endoglucanase component comprised only the 60-amino-acid, C-terminal cellulose-binding domain, suggesting that at least one factor limiting hybrid protein secretion might be the size of the reporter.

L4 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:624994 CAPLUS
DOCUMENT NUMBER: 121:224994
TITLE: A novel **pullulanase** that is thermostable under acid conditions and cloning and expression of the gene encoding it
INVENTOR(S): DeWeer, Philippe; Amory, Antoine
PATENT ASSIGNEE(S): Solvay et Cie., Belg.
SOURCE: Eur. Pat. Appl., 61 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 605040	A1	19940706	EP 1993-203593	19931220
EP 605040	B1	19990811		

R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, PT

BE 1006483	A3	19940913	BE 1992-1156		19921228
BE 1007313	[redacted]	19950516	BE 1993-744	[redacted]	19930715
BE 1007723	[redacted]	19951010	BE 1993-1278	[redacted]	19931119
AT 183236	E	19990815	AT 1993-203593		19931220
ES 2137222	T3	19991216	ES 1993-203593		19931220
FI 9305900	A	19940629	FI 1993-5900		19931228
CN 1090325	A	19940803	CN 1993-121736		19931228
CN 1061089	B	20010124			
JP 06217770	A2	19940809	JP 1993-337202		19931228
CA 2112028	AA	19940629	CA 1993-2112028		19931229
AU 9352759	A1	19940707	AU 1993-52759		19931230
AU 686574	B2	19980212			
US 5721127	A	19980224	US 1995-474140		19950607
US 5721128	A	19980224	US 1995-477630		19950607
US 5731174	A	19980324	US 1995-472293		19950607
US 5736375	A	19980407	US 1995-474545		19950607
US 6074854	A	20000613	US 1997-996733		19971223
AU 9864831	A1	19980730	AU 1998-64831		19980511

PRIORITY APPLN. INFO.:

BE 1992-1156	A	19921228
BE 1993-744	A	19930715
BE 1993-1278	A	19931119
US 1993-174893	B1	19931228
US 1995-472293	A1	19950607

AB A novel **pullulanase** that is heat-stable at acid pHs is obtained from **Bacillus** and the gene encoding it is cloned and expressed for manuf. of the enzyme for processing polysaccharides. The enzyme has a temp. optimum of 55-65.degree. at pH 4.3 and retains >80% of its activity in the pH range 3.8-4.9. An isolate of **Bacillus** deramificans capable of hydrolyzing a pullulan deriv. at 37.degree.; the strain (B. deramificans T89.117D) was not itself heat-tolerant. The enzyme accumulated in the medium and was purified 10-fold (32% yield) from cultures grown on yeast ext./potato starch medium by centrifugation, heat treatment, acetone pptn., and ion-exchange chromatog. The gene was cloned by expression from a Sau3A partial bank in pBR322. The cloned gene was expressed in a **Bacillus** licheniformis host from which the alk. proteinase gene had been **deleted** using either an autonomously replicating or integrating plasmid.

L4 ANSWER 12 OF 14 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1993:504497 BIOSIS
DOCUMENT NUMBER: PREV199396128504
TITLE: The role of the lipoprotein sorting signal (aspartate plus-2) in **pullulanase** secretion.
AUTHOR(S): Poquet, Isabelle; Kornacker, Michael G.; Pugsley, Anthony P. (1)
CORPORATE SOURCE: (1) Unite de Genetique Mol. (CNRS-URA 1149), Inst. Pasteur, 25 Rue du Dr Roux, Paris 75724 Cedex 15 France
SOURCE: Molecular Microbiology, (1993) Vol. 9, No. 5, pp. 1061-1069.
ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The analyses of hybrid proteins and of **deletion** and insertion mutations reveal that the only amino acid at the amino-proximal end of the cell surface lipoprotein **pullulanase** that is specifically required for its extracellular secretion is an aspartate at position +2, immediately after the fatty acylated amino-terminal cysteine. To see whether the requirement for this amino acid is related to its proposed role as a cytoplasmic membrane lipoprotein sorting signal, we used sucrose gradient floatation analysis to determine the subcellular location of **pullulanase** variants (with or without the aspartate residue) that

accumulated in cells lacking the **pullulanase**-specific secretion genes. A non-secr[redacted]ble **pullulanase** variant with a[redacted]rine at position +- 2 cofr[redacted]ctionated mainly with the major [redacted]eak of outer membrane porin. In contrast, most (55%) of a **pullulanase** variant with an aspartate at position +2 cofractionated with slightly lighter fractions that contained small proportions of both outer membrane porin and the cytoplasmic membrane marker NADH oxidase. Only 5% of this **pullulanase** variant cofractionated with the major NADH oxidase peak, while the rest (c. 40%) remained at the bottom of the gradient in fractions totally devoid of porin and NADH oxidase. When analysed by sedimentation through sucrose gradients, however, a large proportion of this variant was recovered from fractions near the top of the gradient that also contained the major NADH oxidase peak. When this peak fraction was applied to a floatation gradient, the **pullulanase** activity remained at the bottom while the NADH oxidase floated to the top. Thus, there is no evidence that lipoproteins that cofractionate with the cytoplasmic membrane under certain conditions are actually associated with the membrane. Instead, the results support our previous proposal that lipoproteins with an aspartate +2 residue are specifically enriched in a distinct domain of the cell envelope that contains material from both the cytoplasmic and the outer membranes. Possible explanations for the requirement for the aspartate residue in **pullulanase** secretion are discussed.

L4 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4

ACCESSION NUMBER: 1992:231648 CAPLUS
DOCUMENT NUMBER: 116:231648
TITLE: An enzyme with type IV prepilin peptidase activity is required to process components of the general extracellular protein secretion pathway of Klebsiella oxytoca
AUTHOR(S): Pugsley, Anthony P.; Dupuy, Bruno
CORPORATE SOURCE: Unite Genet. Mol., Inst. Pasteur, Paris, 75724, Fr.
SOURCE: Mol. Microbiol. (1992), 6(6), 751-60
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The last gene (pulO) of the pul-O **pullulanase** secretion gene operon of Klebsiella oxytoca codes for a protein that is 52% identical to the product of the pilD/xcpA gene required for extracellular protein secretion and type IV plus biogenesis in Pseudomonas aeruginosa. The PilD/XcpA protein is known to remove the first six amino acids of the signal sequence of the type IV pilin precursor by cleaving after the glycine residue in the conserved sequence GF(M)XXXE (where X represents hydrophobic amino acids). This prepilin peptidase cleavage site is present in the products of four genes in the pulC-O operon (PulG, PulH, PulI and PulJ proteins). It is shown here that PulO processes the pulG gene product in vivo. Processing was maximal within 15 s, but expts. in which the expression of pulO was uncoupled from that of the other genes in the secretion operon suggest that processing can also occur post-translationally. The products of two pulG derivs. with internal inframe **deletions** were also processed by PulO, but the three PulG-PhoA hybrids, two PulJ-PhoA hybrids and the single PulH-PhoA hybrid tested did not appear to be processed. Sucrose gradient fractionation expts. showed that both precursor and mature forms of PulG appear to be assocd. with low-d., outer membrane vesicles prepd. by osmotic lysis of sphaeroplasts. Neither the xcpA gene nor the **Bacillus** subtilis gene comC, which is also homologous to pulO and codes for a protein with type IV prepilin activity, can correct the **pullulanase** secretion defect in an Escherichia coli strain carrying all of the genes required for secretion except pulO. Furthermore, neither XcpA nor ComC is able to process prePulG protein in vivo.

L4 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 5

ACCESSION NUMBER: 1990:585484 CAPLUS
DOCUMENT NUMBER: 113:185484
TITLE: Characteristics of thermostable **pullulanase** from **Bacillus** stearothermophilus and the nucleotide sequence of the gene
AUTHOR(S): Kuriki, Takashi; Park, Jonghyun; Imanaka, Tadayuki
CORPORATE SOURCE: Fac. Eng., Osaka Univ., Suita, 565, Japan
SOURCE: J. Ferment. Bioeng. (1990), 69(4), 204-10
CODEN: JFBIEX; ISSN: 0922-338X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Thermostable **pullulanase** was purified to homogeneity on sodium dodecyl sulfate-polyacrylamide gel from the culture supernatant of B. stearothermophilus TRS128. However, multiformity of the **pullulanase** was suggested by activity staining on a pullulan-reactive red plate. The thermostability of the enzyme was tested. In the presence of Ca2+, the optimum temp. of the **pullulanase** was 75.degree., and nearly 100% of the enzyme activity was retained even after treatment at 68.degree. for 60 min. Since the thermostable **pullulanase** gene (pulT) has been cloned, the nucleotide sequence was detd. Although the DNA sequence revealed only one large open reading frame, 2 possible pairs of SD sequence and initiation codon were found in the frame. To analyze the regulatory region, several mutations (**deletion**, insertion and substitution of nucleotides) were introduced in the flanking region of pulT, using site-directed mutagenesis. A putative promoter, SD sequence and initiation codon were inferred. The pulT gene was composed of 1974 bases and 658 amino acid residues (mol. wt. 75,375). The deduced amino acid sequence of the thermostable **pullulanase** exhibited a fairly low homol. with that of the thermolabile **pullulanase** from Klebsiella aerogenes. However, 4 consensus sequences contg. catalytic and(or) substrate binding sites for amylolytic enzymes were also found in the thermostable **pullulanase** and the thermolabile enzyme.

=> log Y